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Occurrence and Antimicrobial Susceptibility Patterns of *Staphylococcus aureus* and *Salmonella* species in Fresh Milk and Milk Products Sold in Zaria and Environs, Kaduna State, Nigeria

Esonu, D. O[1*]., Ismail, S[1]., Ajala. A[1]., Yusuf, S. M.[1] and Otolorin, R.G.[2]

[1]*Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria Kaduna State*
[2]*Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Jos, Jos Plateau State*

[*] *Author for Correspondence: esonu25@gmail.com.*

ABSTRACT

Staphylococcal infections and salmonellosis are important food-borne diseases affecting both humans and animals, and the spread of antibiotic resistant bacteria to humans through the consumption of animal food products remains a challenge. To determine the occurrence and antimicrobial susceptibility pattern of *Staphylococcus aureus* and *Salmonella* in fresh milk and milk products sold in Zaria and environs, Kaduna State, Nigeria, ninety samples were purchased comprising of 49 pasteurized milk, 20 ghee (Clarified butter) and 21 fresh milk samples in Zaria. The Samples were pre-enriched before plating on a suitable selective media. Biochemical and Sugar fermentation tests were used to detect *S. aureus* and *Salmonella* isolates; also, antimicrobial susceptibility test was carried out on positive isolates. The overall prevalence of *S. aureus* and *Salmonella* spp. in fresh milk and milk products (pasteurized milk and ghee) were 31.1% (28/90) and 6.7% (6/90), respectively. Higher prevalence of *S. aureus* was found in fresh milk (38.1%) than in ghee (35%) and pasteurized milk (26.5%). Also, a higher prevalence of *Salmonella* organism was found in fresh milk (9.5%) than in pasteurized milk (6.1%) and ghee (5%). Positive isolates of *S. aureus* showed the highest percentage of antibiotic resistance to oxacillin (100%), then tetracycline (64%) and vancomycin (57.1%), while positive isolates of *Salmonella* showed oxacillin (100%) and vancomycin (100%), followed by tetracycline (33.3%). Most of the isolates displayed resistance to more than three (3) different classes of antimicrobials, indicating multi-drug resistance. Therefore, there is a need for education and public awareness regarding hygienic processing, handling and storage of fresh milk and milk products; thus, the abuse and indiscriminate use of antibiotics in animals should be discouraged.

Keywords: Antimicrobials; Ghee; Fresh milk; Salmonellosis; Staphylococcal infections.

INTRODUCTION

Staphylococcus aureus (*S. aureus*) is a facultative anaerobic Gram-positive bacterium. Majority of *S. aureus* strains are catalase-positive which constitute the pathogenic species (Rasigade and Vandenesch, 2014). It is one of the most important causes of food poisoning worldwide (Spanu *et al*., 2012). Food contamination with *S. aureus* may occur directly from infected food-producing animals or may result from poor hygiene during production processes, or the retail and storage of food (Mdegela *et al*., 2004).

Salmonella is a Gram-negative bacterium, rod-shaped, aerobic or facultative anaerobic, belonging to the Family *Enterobacteriaceae* and it is a primary pathogenic bacterium inhabiting the intestinal tract of humans and animals. *Salmonella* spp. is a significant cause of bacterial contamination of the environment and the food chain (Ponce *et al*., 2008). According to the Centers for Disease Control and Prevention (CDC, 2011); the genus *Salmonella* contains two species, *Salmonella enterica* and *Salmonella bongori*.

Milk is an essential part of the daily diet for growing children and animals. It has been described as a nearly perfect food because it contains the essential nutrients required by the body in appropriate proportions. However, the safety of milk and dairy products with respect to food-borne diseases is a major global issue especially in the developing countries where the production of milk and milk products takes place under poor hygienic, sanitary and agricultural practices (Jordan, 2007). The complex biochemical composition, nutritional values and high-water content render milk an excellent growth medium for both pathogenic and spoilage microorganism (Lawan *et al*., 2012) associated with several disease conditions notably staphylococcosis, salmonellosis, brucellosis, tuberculosis, shigellosis, cholera and host of others (Fagundes *et al.,* 2010; Umaru *et al.,* 2012).

In developing countries, contaminated vegetables, food, water and human-to-human transmission are believed to contribute to the comparatively larger proportion of human cases of staphylococcal and *Salmonella* infection than those in developed countries (Acha and Szyfres, 2003). *Staphylococcus aureus* is a major human pathogen causing a wide range of diseases, such as abscesses, osteomyelitis, necrotizing pneumonia, infective endocarditis, toxic shock syndrome (TSS), bacteremia, septic arthritis, wound infections, pyogenic lesions and sepsis. The organism is a major cause of nosocomial and community-acquired infections and diseases (Umaru *et al*., 2016). Salmonellosis is one of the major zoonotic diseases all over the world with annual estimates of 22 million cases and 200,000 deaths due to typhoid fever and 93.8 million cases of gastroenteritis and 155,000 deaths due to Non-typhoidal *Salmonella*e (NTS) (Majowicz *et al*., 2010).

Antibiotics have been widely used in the treatment of infections in farm animals (Waters *et al*., 2011) and their use has been linked to the spread of antibiotic resistant bacteria to humans through the consumption of animal food products (Marshall *et al*., 2011). In addition, the extensive misuse of antibiotics in all settings has created intense selection pressure, which has resulted in the survival and persistence of resistant strains (Shryock and Richwine, 2010). This poses a challenge to veterinarians, health professionals, and dairy cattle producers due to its negative impact on the response to antimicrobial therapy (Abera *et al*., 2010). Studies carried out in different countries reported increased antimicrobial resistance among *S. aureus* isolates (Thaker *et al*., 2013). There is also increasing resistance to fluoroquinolones by *Salmonella* spp. as reported in Lagos, which is of public health concern (Akinyemi *et al*. 2007). The antimicrobials resistance profile of coagulase positive Staphylococci isolated from ready-to-eat foods and cow milk have been documented in Nigeria (Umaru *et al.,* 2012; Okpo *et al*., 2016; Usman and Mustapha, 2016).

There are so many people still purchasing milk and milk products from the local milk vendors for day-to-day consumption, which in most cases is unpasteurized. People believe that this unpasteurized milk have more nutritive value and lesser cost than pasteurized milk (Adesiyun *et al*., 1998). Hence this may lead to disease outbreaks which can be contracted through milk, if milk were not adequately pasteurized or consumed fresh. Studies have revealed that milk from the local vendors are contaminated with pathogens, and resistance of these organisms to antibiotics is of serious public health concern, thus a need for this study to evaluate the occurrence of *S. aureus* and *Salmonella* species in milk and milk products sold in Zaria, Kaduna State.

MATERIALS AND METHOD

Study Area

The study was conducted in districts of Zaria, Giwa, and Sabon gari Local Government Area, Kaduna State. Kaduna State is located in the North-west geopolitical zone of Nigeria, and the area is known for its hot climate; however, agriculture is the mainstay with about 80% of the people engaging in farming. Another occupation of the people is animal rearing, namely cattle, sheep, goats and pigs (Kaduna State, 2019).

Study Design

This was a cross-sectional study in which fresh milk and milk products were purchased from local vendors within Zaria and its environs, Kaduna State. A total of 90 samples were collected; 49 pasteurized milk, 20 ghee (Clarified butter) and 21 fresh milk from different local vendors from September to October, 2019. Samples were collected based on availability using convenience sampling.

Sample Collection

About 10 ml of milk and milk products were collected from each vendor in sterile sample bottles. All samples were properly labelled and transported in a cool box with ice packs to the Bacterial Zoonoses Laboratory, Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria. The milk samples were processed immediately.

Laboratory Procedures

Each sample was stirred and divided into two parts; one (labelled A) for detection of *Staphylococcus aureus* and the other (labelled B) for detection of Salmonellae.

Processing of Milk and Milk Products for Isolation of *Staphylococcus aureus*

Pre-enrichment

Part A of each sample was pre-enriched with Tryptone Soy Broth (TSB) at 9:1 ratio (9 parts of TSB and 1 part of the sample) under aseptic condition, labelled and incubated for 24 hours to enhance microbial growth.

Selective Plating

Using a sterile wire loop, a loop-full of incubated Tryptone Soy Broth containing the sample was streaked on Mannitol Salt Agar (MSA) plates. The plates were labelled and incubated at 37° C and examined after 24 hours for growth and change in the colour of the medium. After incubation at 37°C, golden-yellow colonies (denoting mannitol fermentation) that were medium size to large, spherical, raised, smooth and appearing in clusters were selected as presumptive *S. aureus*. The isolates were then stored on nutrient agar slants and incubated at 37°C for 24 hours. The isolates were then stored at 4°C until required.

Biochemical Tests

Staphylococcus aureus isolates identification was done by colony morphology, Gram staining and biochemical tests (catalase, coagulase). All suspected cultures of *Staphylococcus* species were subjected to Gram's stain and observed under a light microscope for Gram's reaction, size, shape, and cell arrangements. The Gram-stained smears from typical colonies that showed Gram-positive cocci occurring in bunches, grapelike or irregular clusters were taken as presumptive *Staphylococcus* species.

Identification by Sugar Fermentation Test

Andrade's peptone water was used as the pH indicator. Equal grams of sugars and Andrade's peptone water to be used were measured using a weighing balance, and media was prepared according to the manufacturer's specification. Sugars used include sucrose, mannitol, lactose, and maltose in which *Staphylococcus aureus* ferment all the sugars and change the pH of the media with the production of pink coloration.

Processing of Milk and Milk Products for *Salmonella* Isolation

Pre-enrichment

The other part (Part B) of each sample were pre-enriched with Rappaport Vassiliadis broth at 9:1 ratio (9 parts of Rappaport and 1 part of sample).

Selective Plating

Using a sterile wire loop, a loop-full of incubated Rappaport -Vassiliadis containing the sample was streaked on *Salmonella Shigella* Agar (SSA) plates. The plates were labelled and incubated at 37°C and examined after 24 hours for growth. The colonial morphology on the plate was then appraised and those colonies that were colourless with black centers indicating non-lactose fermenters were stored on Nutrient Agar slants in sample bottles and incubated at 37° C for 24 hours and kept in the refrigerator pending biochemical characterization.

Biochemical Tests

Suspected *Salmonella* isolates from the nutrient agar slant were subjected to biochemical tests based on indole production, H_2S production, motility with Sulfide Indole Motility (SIM) medium, Citrate utilization with Simmons citrate, Methyl Red (MR) and Voges-Proskauer (VP) using MR-VP medium and Urease production, Presumptive colonies were transferred to tubes of Triple Sugar Iron (TSI) agar, Simmon's Citrate, Urea, Methyl Red, Voges Proskauer and incubated at 37° C for 24 hours. Confirmed isolates were stored on nutrient agar slants at 4° C for further studies. All isolates that were typical of *Salmonella* spp. were tested, and substrates were considered to belong to the genus *Salmonella*. Typical *Salmonella* reactions such as indole negative, methyl red positive, Voges-Proskauer negative, citrate positive, motile in motility medium and produces H_2S and urea.

Identification by Sugar Fermentation Test

Andrade's peptone water was used as the pH indicator. An equal gram of sugars and Andrade's peptone water to be used was measured using a weighing balance and media was prepared according to the manufacturer's specification. Sugars used include sucrose, mannitol, glucose, lactose, and maltose in which *Salmonella* spp. ferment all the sugars and change the pH of the media.

Antimicrobial Susceptibility Test

Antimicrobial susceptibility tests were performed on all the *S. aureus,* and *Salmonella* isolates using disk diffusion assay as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2016). The correctly identified organisms were used for subculture on Mueller-Hinton agar plate. Panels of selected antimicrobials commonly used in the empirical treatment of *S*taphylococcal and *Salmonella* infections informed the choice of antibiotics used. Antibiotics used were: Vancomycin (30µg) a glycopeptide, Chloramphenicol (30µg) an amphenicol, Tetracycline (30µg) a tetracycline, Ceftriaxone (30µg) a third-generation cephalosporin, Amikacin (30µg) an aminoglycoside, Gentamycin (10µg) an aminoglycoside, Imipenem (10µg) a carbapenem, Nitrofurantoin (300µg) a nitrofuran, Oxacillin (1µg) a penicillin and Enrofloxacin (5µg) a fluoroquinolone. Their effects on growth of *S. aureus* and *Salmonella* were evaluated by harvesting young and overnight pure cultures after 24 hours of incubation using a sterile inoculating loop to touch four or five colonies of the pathogen growing on agar and then used to inoculate a tube containing 5 ml of sterile normal saline. The solution was mixed thoroughly and poured into Mueller-Hinton agar plate and spread by tilting to cover the entire plate and poured back into the tube. After the agar surface dried for 5 minutes, the appropriate antibiotics were placed at equidistant on it using multi-antibiotic disc dispenser. The test plates were then incubated at 37° C for 24 hours and then removed and inspected for satisfactory growth of *S. aureus* and *Salmonella* species. The diameter of the zones of inhibition for each antibiotic was read as recommended by the Clinical and Laboratory Standards Institute (CLSI) standard (CLSI, 2016).

Data Analysis

Data collected was analyzed using SPSS software (version 20, SPSS Inc., IBM, USA 2016). Chi-square was used to test for association between *S. aureus and Salmonella* and factors such as markets, LGAs and products sampled. $P \leq 0.05$ was considered to be statistically significant. Data obtained were further expressed as frequencies and percentages, and the prevalence of *S. aureus* and *Salmonella* in the milk and milk products were determined.

RESULTS

The overall prevalence of *S. aureus* and *Salmonella* spp. in fresh milk and milk products (pasteurized milk and ghee) within selected markets in Zaria and environs were 31.1% (28/90) and 6.7% (6/90) respectively. Higher prevalence of *S. aureus* was found in fresh milk (38.1%) than in ghee (35%) and pasteurized milk (26.5%). Also, a higher prevalence of *Salmonella* organism was found in fresh milk (9.5%) than in pasteurized milk (6.1%) and ghee (5%). (Table 1).

From all the three local government areas (LGA) investigated for presence of *S. aureus* in fresh milk and milk products, Giwa (38.1%) had the highest prevalence of contaminated samples when compared with Sabon gari (32%) and Zaria (27.3%). Higher prevalence of *Salmonella* organism was observed in Sabon gari (12%) than in Zaria (2.3%) and Giwa (9.5%) (Table 1).

Gwargwaji market (40%) in Giwa LGA has the highest prevalence of *S. aureus*, Sabo market (14.3%) in Sabon gari LGA had the lowest, while the occurrence of *Salmonellae* based on market was observed to be higher in Samaru market (16.7%) followed by Gwargwaji (10%) and Shika (9.5%). No

Salmonella organisms were observed in Tudun wada and Sabo market.

Antimicrobial-resistance patterns of the positive isolates of *S. aureus* in Table 2, showed the highest percentage of resistance to oxacillin (100%) followed by tetracycline (64.3%), vancomycin (57.1%), and ceftriaxone (39.3%). 21.4% of positive isolates were resistant to enrofloxacin and chloramphenicol, 17.9% resistant to amikacin, 14.3% resistant to nitrofurantoin and gentamicin, and 10.7% resistant to imipenem. Positive isolates of salmonella spp. in this study showed the highest percentage of antimicrobial resistance to oxacillin (100%) and vancomycin (100%), followed by tetracycline (33.3%). Also, 16.7% of the isolates were resistant to ceftriaxone, chloramphenicol and amikacin. However, none of the *Salmonellae* isolates showed resistance to gentamicin, enrofloxacin, nitrofurantoin, and imipenem (Table 3). Of the 28 *S. aureus* isolates, 24 (85.7%) displayed resistance to two (2) or more antimicrobials, whereas twenty (71.4%) isolates showed resistance to more than three (3) different classes of antimicrobials (multi-drug resistance) (Table 4). While out of *Salmonella* isolates, 3 (50%) displayed resistance to more than three (3) different classes of antimicrobials (multi-drug resistance) (Table 5).

DISCUSSION

A higher prevalence of *S. aureus* was observed in this study when compared with previous studies in which 12.6% and 15.3% were reported by Umaru *et al.* (2012) and Umaru *et al.* (2016) respectively, in the same study areas.

Table 1: Prevalence of *S. aureus* and *Salmonella* spp. in fresh milk and milk products sold in Zaria and Environs

Factors		Number Examined	Number positive (%)	
			Staphylococcus aureus*	***Salmonella* spp.****
[1]**Products**	**Fresh milk**	21	8 (38.1)	2 (9.5)
	Past. milk	49	13 (26.5)	3 (6.1)
	Ghee	20	7 (35)	1 (5)
[2]**LGAs**	**Sabon gari**	25	8 (32)	3 (12)
	Zaria	44	12 (27.3)	1 (2.3)
	Giwa	21	8 (38.1)	2 (9.5)
[3]**Markets**	**Dan Magaji**	11	3 (27.3)	0 (0.0)
	Gwargwaji	10	4 (40)	1 (10.0)
	Tudun Wada	23	5 (21.7)	0 (0.0)
	Samaru	18	7 (38.9)	3 (16.7)
	Sabo	7	1 (14.3)	0 (0.0)
	Shika	21	8 (38.1)	2 (9.5)
Total		**90**	**28 (31.1)**	**6 (6.7)**

[1](*χ^2= 1.099; p= 0.577)
[1](**χ^2= 1.159; p=0.560)
[2](*χ^2= 0.790; p= 0.674)
[2](**χ^2= 2.784; p= 0.249)
[3](*χ^2= 3.298; p= 0.654)
[3](**χ^2= 6.276; p= 0.280)

Table 2: Antibiotic susceptibility profile of positive isolates of *S. aureus* from fresh milk and milk products sold in Zaria and Environs

Antibiotics	Susceptible (%)	Intermediate (%)	Resistance (%)
Tetracycline	9 (32.1)	1 (3.6)	18 (64.3)
Ceftriaxone	5 (17.9)	12 (42.9)	11 (39.3)
Gentamicin	24 (85.7)	0 (0.0)	4 (14.3)
Enrofloxacin	20 (71.4)	2 (7.1)	6 (21.4)
Chloramphenicol	20 (71.4)	2 (7.1)	6 (21.4)
Oxacillin	0 (0.0)	0 (0.0)	28 (100)
Vancomycin	12 (42.9)	0 (0.0)	16 (57.1)
Amikacin	18 (64.3)	5 (17.9)	5 (17.9)
Imipenem	25 (89.3)	0 (0.0)	3 (10.7)
Nitrofurantoin	24 (85.7)	0 (0.0)	4 (14.3)

Table 3: Antibiotic susceptibility profile of positive isolates of *Salmonella* spp. from fresh milk and milk products sold in Zaria and Environs

Antibiotics	Susceptible (%)	Intermediate (%)	Resistance (%)
Tetracycline	4 (66.7)	0 (0.0)	2 (33.3)
Ceftriaxone	5 (83.3)	0 (0.0)	1 (16.7)
Gentamicin	6 (100)	0 (0.0)	0 (0.0)
Enrofloxacin	6 (100)	0 (0.0)	0 (0.0)
Chloramphenicol	5 (83.3)	0 (0.0)	1 (16.7)
Oxacillin	0 (0.0)	0 (0.0)	6 (100)
Vancomycin	0 (0.0)	0 (0.0)	6 (100)
Amikacin	5 (83.3)	0 (0.0)	1 (16.7)
Imipenem	6 (100)	0 (0.0)	0 (0.0)
Nitrofurantoin	4 (66.7)	2 (33.3)	0 (0.0)

Table 4: Antimicrobial resistance patterns of positive isolate of *S. aureus* from fresh milk and milk products sold in Zaria and Environs

No. of Antibiotics	Resistance Pattern	No. of Isolates (%)
1	Oxa	4 (14.3)
2	Cef, Oxa	1 (3.6)
2	Enro, Oxa	2 (7.1)
2	Tet, Oxa	1 (3.6)
3	Tet, Oxa, Van	5 (17.9)
3	Oxa, Van, Ami	1 (3.6)
3	Tet, Chlo, Oxa	1 (3.6)
3	Cef, Oxa, Van	2 (7.1)
3	Tet, Cef, Oxa	2 (7.1)
4	Tet, Cef, Oxa, Van	2 (7.1)
4	Tet, Chlo, Oxa, Van	1 (3.6)
4	Tet, Oxa, Van, Nitro	1 (3.6)
5	Tet, Oxa, Van, Ami, Nitro	1 (3.6)
6	Tet, Cef, Gen, Enro, Chlo, Oxa	1 (3.6)
9	Tet, Cef, Gen, Enro, Chlo, Oxa, Van, Ami, Imip	1 (3.6)
10	Tet, Cef, Gen, Enro, Chlo, Oxa, Van, Ami, Imip, Nitro	2 (7.1)

Van = Vancomycin, Chlo = Chloramphenicol, Tet = Tetracycline, Cef = Ceftriaxone, Ami = Amikacin, Gen = Gentamicin, Imip = Imipenem, Nitro = Nitrofurantoin, Oxa = Oxacillin, Enro = Enrofloxacin

Table 5: Antimicrobial resistance patterns of positive isolate of *Salmonella* spp. from fresh milk and milk products sold in Zaria and Environs.

No. of Antibiotics	Resistance Pattern	No. of Isolates (%)
2	Oxa, Van	3 (50)
3	Tet, Oxa, Van	1 (16.7)
3	Oxa, Van, Ami	1 (16.7)
5	Tet, Cef, Chlo, Oxa, Van	1 (16.7)

Van = Vancomycin, Chlo = Chloramphenicol, Tet = Tetracycline, Cef = Ceftriaxone, Ami = Amikacin, Oxa = Oxacillin

This rate was also higher than that recorded in previous studies in Algeria 26% (André *et al.,* 2008) and 21% in Iran (Nazari *et al.,* 2014). The prevalence of *Salmonella* gotten from this study is of public health significance as no *Salmonella* was expected to be present in food meant for consumption. This prevalence complies with that of Karshima *et al.* (2013) who also found a prevalence of 6.4% from fresh milk and fermented milk in Kanam, Plateau State, while Mhone *et al.* (2012), reported zero prevalence of *Salmonella* spp. in fresh milk from selected farms in Zimbabwe. These differences could be attributed to the fact that most of these researchers carried out their samples on farms, while our study was interested in the milk and milk products sold by local vendors which may have undergone several means of unhygienic transport, handling and storage as these have been reported to be factors that predisposes milk to contamination with pathogens (Akram *et al*., 2013).

In this study, fresh milk showed a higher contamination rate compared to butter ghee and pasteurized milk. This could be associated with unhygienic milking and poor handling practices that could lead to poor quality of milk. Pasteurized milk and ghee had undergone some form of heat treatment which thus, lowers the bacterial load. This indicates that pasteurization and fermentation eliminated most of the organisms, as reported by Umaru *et al.* (2012).

Gwargwaji market showed a high contamination rate, and this could be attributed to the close proximity of the local vendors to the very busy federal highway which is most times concentrated with heavy dust particles exposing these products to varieties of microorganisms. Some of the selling points in this market were also located in or close to motor parks and road-side shops which is overcrowded putting the products at a high risk of contamination.

Yakubu *et al*. (2020) reported antimicrobial resistance of *S. aureus* to tetracycline (44.4%) while no resistance was observed with gentamicin, vancomycin, chloramphenicol and imipenem. Those obtained by Daka *et al.* (2012) were vancomycin (38.5%), ceftriaxone (23.5%), and ciprofloxacin (23.5%). The findings from this study show that the current situation is significantly different, with regards to oxacillin (100%), tetracycline (64.29%), vancomycin (57.14%), ceftriaxone (39.28%) enrofloxacin and chloramphenicol (21.43%). This could be attributed to the indiscriminate use of antibiotics in animals and animal husbandry. Oxacillin (penicillin) and tetracycline resistance observed could be attributed to these drugs been among the readily available antimicrobials used as growth promoters and routine prophylaxis in livestock management in Nigeria (Olatoye, 2010).

The findings from this study showed that isolates of *S. aureus* displayed resistance to more than three (3) different classes of antimicrobials, thus multi-drug resistance. This finding is in agreement with the findings of Umaru *et al*. (2013) and Anueyiagu and Isiyaku, (2015) who reported cases of multidrug resistance among *S. aureus* isolated from dairy products in Zaria and Jos respectively. Multidrug resistance in *S. aureus* may be due to the spread of mobile genetic elements like plasmids, transposons, and integrons that may confer resistance to numerous antimicrobial agents (Zhao *et al*., 2001).

The antibiotic susceptibility patterns of *Salmonella* spp. showed that the isolates from milk and milk products were resistant to commonly used antimicrobials, but all the isolates were sensitive to gentamicin, enrofloxacin, imipenem and nitrofurantoin. This finding is similar to that described by Chen *et al*. (2013) who carried out antibiotic sensitivity on *Salmonella* isolates in China and reported that all isolates were sensitive to gentamicin and ciprofloxacin. The probable reason of multi-drug resistance with 100% resistance to Oxacillin and Vancomycin may also be due to spread of mobile genetic elements of these pathogens in addition to the indiscriminate use of these antimicrobials by farmers in preventing or treating certain diseases of their animals (Zhao *et al*., 2001).

The presence of *S. aureus* and *Salmonella* in milk samples purchased from various local vendors indicates milk may be contaminated due to unhygienic handling or these samples are being sold to these consumers directly without any processing. These practices may lead to serious public health issues like foodborne intoxication, or transfer of antibiotic-resistant *S. aureus* to the human population.

Conclusion

In this study, *S. aureus* isolates were recovered from 31.11% (28/90) of fresh milk and milk product samples collected from local vendors. The study also demonstrated the presence of *Salmonella* isolates (6.7%) in milk and milk products with higher contamination in fresh milk than pasteurized milk. This may indicate that there is a possibility of potential public health threat through consumption of contaminated milk and milk products. Twenty (71.4%) *S. aureus* isolates presented multiple drug resistance for more than three drugs. This study has also shown that the drug of choice for treatment of salmonellosis includes: gentamicin, imipenem and enrofloxacin due to high level of susceptibility of the organism to the drugs.

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Conflict of Interest

The authors have no conflict of interest to declare.

Author's Contribution

DOE, SMY and RGO designed the research, DOE, SI and AA carried it out while DOE, SI, AA and SMY were involved in the analyses, writing and proof reading of the manuscript.

REFERENCES

Abera, M., Demiel, B., Aragaw, K., Regassa, F. and Regassa, A (2010). Isolation and identification of *Staphylococcus aureus* from bovine mastitis milk and their drug resistance pattern in Adama town, Ethiopia. *Journal of Veterinary Medicine and Animal Health*, 3:29-34.

Acha, P.N. and Szyfres, B. (2003). Zoonoses and communicable diseases common to Man and Animals 3rd edition, Vol.1, published by Pan American Health Organization, Pp. 233-234.

Adesiyun, A.A., Webb, L.A., and Romain, H.T. (1998). Prevalence and characteristics of *Staphylococcus aureus* strains isolated from bulk and composite milk and cattle handlers. *Journal of Food* Protection 61:629–632.

Akinyemi, K.O., Bamiro, B.S. and Coker, A.O. (2007). Salmonellosis in Lagos, Nigeria: incidence of *Plasmodium falciparum*-associated co-infection, patterns of antimicrobial resistance, and the emergence of reduced susceptibility to fluoroquinolones. *Journal of Health Population and Nutrition*., 25(3): 351.

Akram, N., Chaudhary, A.H., Ahmed, S., Ghuma, M.A., Nawaz, G. and Hussain, S. (2013). Isolation of bacteria from mastitis affected bovine milk and their antibiogram. *European Journal of Veterinary Medicine,* 2(1): 38-46.

André, M.C.D., Campos, M.R.H., Borges, L.J., Kipnis, A., Pimenta, F.C. and Serafini, A. B. (2008). Comparison of *Staphylococcus aureus* isolates from food handlers, raw bovine milk and minas frescal cheese by antibiogram and pulsed-field gel electrophoresis following Small digestion. *Food Control,* 19: 200-207.

Anueyiagu, K.N. and Isiyaku, A.W. (2015). Isolation and identification of *Staphylococcus aureus* from bovine milk and its antibiotic susceptibility. *International Journal of Livestock Production*., 6(6): 74-77

Centers for Diseases Control and Prevention (CDC) (2011). Surveillance System Overview: National *Salmonella* Surveillance, Pp. 1-12.

Chen, H.M., Wang, Y., Su, L.H. and Chiu, C.S. (2013). Non-typhoid *Salmonella* Infection: Microbiology, clinical features, and antimicrobial therapy. *Journal of Pediatric Neonatal and Individual. Medicine*., 54: 147-152.

Clinical and Laboratory Standards Institute (CLSI) (2016): Performance standards for antimicrobial susceptibility testing; Twenty-sixth informational supplement. CLSI document M100-S26. Wayne, PA: Clinical and Laboratory Standards Institute.

Daka, D., Solomon, G. and Dawit, Y. (2012). Antibiotic-resistance *Staphylococcus aureus* isolated from cow's milk in the Hawassa area, South Ethiopia. *Annals of Clinical Microbiology and Antimicrobials*., 11:26-32.

Fagundes, H., Barchesi, L., Filho, A.N., Ferreira, L.N. and Oliveira, C.A.F. (2010). Occurrence of *Staphylococcus aureus* in raw milk produced in dairy farms in São Paulo state, Brazil. *Brazilian Journal of Microbiology*., 41 (2): 376–380.

Feng, Y., Chen, C.J., Su, L-H., Hu, S., Yu, J. and Chiu, C-H. (2008). Evolution and pathogenesis of *Stapphylococcus aureus*: lessons learned from genotyping and comparative genomics. *FEMS Microbiology Letters*, 32: 23-37.

Kaduna State Government (2019). Alias: Centre of Education. Retrieved from http://www.kdsg.gov.n g.

Karshima, N.S., Pam, V.A., Bata, S.I., Dung, P.A. and Paman, N.D. (2013). Isolation of *Salmonella* species from Milk and Locally Processed Milk Products Traded for Human Consumption and Associated Risk factors in Kanam, Plateau State, *Nigerian Journal of Animal Production Advances*, 3 (3): 69.

Lawan, M.K., Abdulsalawu, F.O., Suleiman, A., Aluwong, T and Yaqub, L.S. (2012). Microbial assessment of bulk of milk before and after pasteurization in two different Dairy Farms in Zaria, Nigeria. *International Journal of Dairy Science*, 7 (4): 103-108.

Majowicz, S.E., Musto, J., Scallan, E., Angulo, F.J., Kirk, M., O'Brien, S.J., Jones, T.F., Fazil, A. and Hoekstra, R.M. (2010). The global burden of non-typhoidal *Salmonella* gastroenteritis. *Clinical Infectious Diseases*., 50(6): 882-9.

Marshall, B.M. and Levy, S.B. (2011). Food animals and antimicrobials: Impacts on human health. *Clinical Microbiology Review*., 24:718-33.

Mdegela, R.H., Kusiluka, L.J., Kapaga, A.M., Karimuribo, E.D., Turuka, F.M., Bundala, A. (2004). Prevalence and determinants of mastitis and milk-borne zoonoses in small holder dairy farming sector in Kibaha and Morogoro districts in Eastern Tanzania. *Journal of Veterinary Medicine Series B*, 51 (3):123-8.

Mhone, T.A., Matope, G. and Saidi, P.T. (2012). Detection of *Salmonella* spp., *Candida albicans*, *Aspergillus* spp., and Antimicrobial Residues in Raw and Processed Cow Milk from Selected Smallholder Farms of Zimbabwe. *Veterinary Medicine International* 2012(3): 301902

Nazari, R., Godarzi, H., Baghi, F.R., and Moeinrad, M. (2014). Enterotoxin gene profiles among *Staphylococcus aureus* isolated from raw milk. *Iranian Journal of Veterinary Research*., 15(4): 409-412.

Okpo, N.O., Abdullahi, I.O., Whong, C.M.Z. and Ameh, J.B. (2016). Occurrence and antibiogram of Staphylococcus aureus in dairy products consumed in parts of Kaduna State, Nigeria. *Bayero Journal of Pure and Applied Sciences,* 9(2): 225-229.

Olatoye, I.O. (2010). The incidence and antibiotics susceptibility of *Escherichia coli* O157:H7 from beef in Ibadan Municipal, Nigeria. *African Journal of Biotechnology*, 9(8): 1196- 1199.

Ponce, E., Khan, A. A., Cheng, C.M., Summage-West, C. and Cerniglia, C.E., (2008). Prevalence and characterization of *Salmonella* enteric serovar Weltevreden from imported seafood. *International Journal of Food Microbiology*, 25: 29-35.

Rasigade, J.P., Vandenesch, F. (2014). Staphylococcus aureus: a pathogen with still unresolved issues. *Infection Genetics and Evolution*, 21: 510-4.

Shryock, T. R. and Richwine, A. (2010). The interface between veterinary and human antibiotic use. *Annals of the New York Academy of Sciences*., 1213: 92-105.

Spanu, V., Spanu, C., Virdis, S., Cossu, F., Scarano, C., De Santis E., *et al.* (2012). Virulence factors and genetic variability of *Staphylococcus aureus* strains isolated from raw sheep's milk cheese. *International Journal of Food Microbiology*., 153: 53-7.

Thaker, H.C., Brahmbhatt, M.N. and Nayak, J.B. (2013). Isolation and identification of *Staphylococcus aureus* from milk and milk products and their drug resistance patterns in Anand, Gujarat. *Veterinary World*, 1:10-3.

Umaru, G.A., Kabir, J., Umoh, V.J., Bello, M. and Kwaga, J.K.P. (2012). Prevalence and Antibiogram of coagulase positive staphylococci isolated from fresh and fermented milk In Zaria and Kaduna, *Nigerian Journal of Veterinary and Applied Scien ces*., 2: 1-7.

Umaru, G.A., Kabir, J., Umoh, V.J., Bello, M. and Kwaga, J.K.P. (2013). Methicillin-resistant *Staphylococcus aureus* (MRSA) in fresh and fermented milk in Zaria and Kaduna, Nigeria. *International Journal of Drug Research and Technology*, 3(3): 67-75.

Umaru, G.A., Kwaga, J.K.P., Bello, M., Raji, M.A. and Maitala, Y.S. (2016). Antibiotic resistance of *Staphylococcus aureus* isolated from Fresh cow milk in settled fulani herds in Kaduna state, Nigeria. *Bulletin of Animal Health and Production in Africa*., 64: 173-182.

Usman, R.Z. and Mustapha, B.M. (2016). Isolation and identification of methicillin-resistant *Staphylococcus aureus* (MRSA) from traditionally fermented milk 'nono' and yoghurt in Kaduna metropolis. *Food Science and Quality Management,* 2(2): 1- 21.

Waters, A.E., Contente-Cuomo, T., Buchhagen, J., Liu, C.M., Watson, L., Pearce, K., *et al.* (2011). Multidrug-resistant *Staphylococcus aureus* in US meat and poultry. *Clinical Infectious Diseases*.,52: 1227-30.

Yakubu, A., Abdullahi, I.O., Whong, C.Z. and Olayinka, B. (2020). Prevalence and antibiotic susceptibility profile of *Staphylococcus aureus* from milk and milk products in Nasarawa State, Nigeria. Sokoto *Journal of Veterinary Science*., 18(1): 1- 12.

Zhao, S., White, D.G., Ge, B., Ayers, S., Friedman, S., English, L., Wagner, D., Gaines, S. and Meng, J. (2001). Identification and characterization of integron- mediated antibiotic resistance among shiga toxin- producing *Escherichia coli* isolates. *Journal of Applied and Environmental Microbiology*, 67(4): 1558- 1564.