JOURNAL OF VETERINARY AND APPLIED SCIENCES
2012 VOL. 2(1): 1 - 7
Manuscript No. JVAS/2012/009; Received: 20/05/2012; Accepted: 12/11/2012
Published by: Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria

PREVALENCE AND ANTIBIOGRAM OF COAGULASE POSITIVE STAPHYLOCOCCI ISOLATED FROM FRESH AND FERMENTED MILK IN ZARIA AND KADUNA, NIGERIA

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ABSTRACT

This study was conducted to determine the prevalence and antibiogram of coagulase positive staphylococci in fresh and fermented milk. A total of 47 (12.6%) coagulase positive staphylococci were detected from 372 milk samples. Bulk milk recorded the highest occurrence (50 %) followed raw milk (7 %), pasteurized milk (5.9%) and 'kindirmo' (5.0%). No staphylococci were detected in the yogurt samples examined. There were no significant differences (P > 0.05) between the occurrences of staphylococci among the types of samples examined. The resistance profile of the isolates were as follows: penicillin (100%), oxacillin (46.8%), amoxicillin (44.8%), sulphamethoxazole/trimethoprim (6.4%), chloramphenicol (4.3%) and amikacin (2.1%). None of the coagulase positive staphylococci was resistant to ciprofloxacin. Thirty seven (78.7%) of the isolates exhibited multi-drug resistant pattern with tetracycline- penicillin (53.2%), tetracycline- penicillin- erythromycin (31.9%) and tetracycline- penicillin-erythromycin-oxacillin-vancomycin (25.5%). Following the high prevalence and multi-drug resistant nature of the coagulase positive staphylococci from this study, proper pasteurization and fermentation of milk prior to human consumption is recommended.

***Keywords*:** Prevalence, Antibiogram, Coagulase Positive Staphylococci, Milk.

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ISSN: 2315 - 6856

INTRODUCTION

Milk is a nutrient fluid produced by the mammary gland of many mammals for the nourishment of their young [1], and accounts for 16% of the total value of all food products produced from livestock in Sub-Saharan Africa [2]. Fresh milk and fermented products are rich nutritionally, but they constitute a good media for multiplication of microbes such as *Staphylococcus*, *Salmonella*, *Mycobacterium* and *Shigella* organisms. Milk that is contaminated with pathogenic organisms at the site of production presents a hazard to human health.

Several food types have been implicated in staphylococcal food poisoning; and these include milk and cream, butter, cheese, sausage, salad and cooked meals [3]. The foods that are most involved in staphylococcal food poisoning differ widely from one country to another. In the United Kingdom, for example, 53% of the staphylococcal food poisonings reported between 1969 and 1990 were due to meat products-based dishes, especially ham; 22% of the cases were due to poultry and poultry-based meats; 8% were due to milk products; 7% to fish and shell fish and 3.5% to eggs [3].

Staphylococcus aureus are common commensal microorganisms and opportunistic pathogens in humans and animals [4]. About 20% of the population are long time carriers with the organism commonly colonizing the skin and nasal passages [5,6,7].

Milk is contaminated with staphylococci from udder and skin of dairy animals which are sometimes infected from the source. The principal health hazard of contamination of milk with staphylococci lies in the fact that some strains produce enterotoxin that can cause acute food poisoning. Although the organisms are usually destroyed at pasteurization temperature, the pre-formed exotoxin is heat resistant.

In Nigeria, the presence of *S. aureus* in fresh and fermented milk; and foods has been documented with prevalence of 30 - 99% [8,9,10,11,12,13,14,15]. This study was conducted to provide current information on the prevalence of staphylococci in raw and fermented milk, their antibiotic resistance pattern and also the efficacy of pasteurization and fermentation in eliminating the organisms.

MATERIAL AND METHODS

The study was carried out in Kaduna and Zaria, Kaduna State, Nigeria. Settled Fulani cattle herds supplying milk to two peri-urban dairy groups namely MILCOPAL in Kaduna and NAPRI in Zaria were covered. The herds were visited during milking time, where 5ml of composite fresh milk samples were collected directly from milking cows and placed into sterile sample bottles. Bulk fresh milk samples were collected after all the milk has been collected and pooled before transporting to the plants. Milk products (Pasteurized milk and yogurt) were collected from the processing plants and 'kindirmo' (locally fermented milk) from the villages. All the samples collected were placed on ice and transported to the laboratory for analysis.

Isolation of staphylococci

All the samples were plated on Baird Parker medium (Oxoid, Basingstoke, England), and incubated aerobically at 37^{o} C for 24 hours [16]. After incubation, the colonies (which were typically grey-black with narrow white margins surrounded by zone of clearing) were purified on fresh media and stocked on nutrient agar slants prior to identification [16]. The isolates were identified using colony morphology, gram staining characteristics and biochemical tests namely: catalase, coagulase, DNase and sugar fermentation [17,18].

Antimicrobial Susceptibility and Resistance Tests

Antimicrobial resistance was performed by Kirby- Bauer disk diffusion method as recommended by Clinical Laboratory Standards Institute (CLSI) [19, 20, 21]. All isolates were grown in Brain Hearth

infusion broth (Biotech Laboratories, United Kingdom) and incubated at 37^{o} C for 6 hours until the turbidity of 0.5 McFarland standards was achieved. The isolates were then swabbed onto Muller Hinton agar (Amershan, England) and the antimicrobial discs applied. The isolates were tested against twelve (12) antibiotics with the following concentrations: amikacin (30 µg), amoxicillin (30 µg), chloramphenicol (12 µg), ciprofloxacin (5 µg), erythromycin (5 µg), gentamycin (10 µg), methicillin (10 µg), oxacillin (1 µg), penicillin (10 IU), sulphamethoxazole/trimethoprim (5 µg), tetracycline (30 µg) and vancomycin (30 µg) (Oxoid, England). Zones of inhibition (ZI) were measured and recorded after 24 hours of incubation at 35^{o} C, and were interpreted according to the guidelines of the CLSI [21]. *S. aureus* ATCC 3359 strain was used as a positive control.

DATA ANALYSIS

Data obtained from the study were analyzed statistically using Statistical Package for Social Sciences (SPSS) Version 13 software. Frequencies were obtained and percentages for study variables were calculated. Chi-square and Fisher's exact tests at 5% level of confidence were used to perform categorical comparisons and determination of significance. A P value < 0.05 was considered significant for all comparisons.

RESULTS

Three hundred and seventy two milk samples were collected (196 were from settled Fulani herds supplying MILCOPAL Dairy Plant in Kaduna and 176 were from those supplying NAPRI Dairy Plant in Zaria). The samples comprised of raw milk (n= 300), bulk milk (n= 18), pasteurized milk (n=17), yogurt (n= 17) and 'kindirmo' (locally fermented milk) (n= 20).

Of the 372 milk samples examined, 47 (12.6%) were coagulase positive (Table 1). Kaduna recorded a higher isolation rate (13.8%) than Zaria (11.4%) with no significant difference (P > 0.05) between the two locations. Among the different types of milk samples examined, bulk milk had the highest occurrence of 50 %, followed by raw milk (12%), pasteurized milk (5.9 %) and 'kindirmo' (locally fermented milk) (5.0 %) each respectively (Table 2). No significant difference exist between the occurrences among the different samples examined (P > 0.05). No *S. aureus* was isolated from any of the yogurt sample examined (Table 1).

Table 1. Prevalence of Staphylococci in fresh and fermented milk samples examined at Kaduna and Zaria, Nigeria.

Location	No. of samples	No. (%) of staphylococcal isolates	
		Coagulase-negative	Coagulase-positive
All samples	372	80 (21.5)	47 (12.6)
Location			
Kaduna	196	49 (25.0)	27 (13.8)
Zaria	176	31 (17.6)	20 (11.4)
Sample type			
Raw milk	300	75 (25.0)	36 (12.0)
Bulk milk	18	5 (27.8)	9 (50.0)
Pasteurized milk	17	0	1 (5.9)
Yogurt	17	0	0
Kindirmo	20	0	1 (5.0)

Antibiotic Resistance and Susceptibility Profiles

Table 2 gives the overall resistance and susceptibility profiles of the 47 coagulase positive staphylococci. The highest resistant was demonstrated against penicillin (100%), oxacillin (46.8%), and amoxicillin (44.7%), while the least was demonstrated against amikacin (2.1%), chloramphenicol (4.3 %) and sulphamethoxazole/trimethoprim (6.4 %). None of the isolates was resistant to ciprofloxacin (Tables 2).

The multiple resistance patterns of the 47 coagulase positive staphylococci are shown in Table 3. Thirty seven (78.7%) of the coagulase positive isolates were resistant to more than one of the antibiotics tested. The most common multiple resistance pattern encountered were tetracycline-penicillin (53.2%), tetracycline-penicillin-erythromycin (31.9%) and tetracycline-penicillin-erythromycin-oxacillin-vancomycin (25.5%).

Table 2. Antibiogram of the 47 coagulase-positive staphylococcal isolates in fresh and fermented milk in Kaduna and Zaria, Nigeria.

Antibiotics	Antibiogram [No. (%)]	
	Resistant	Susceptible
Amikacin	1 (2.1)	46 (97.9)
Amoxicillin	21 (44.7)	26 (55.3)
Chloramphenicol	2 (4.3)	45 (96.7)
Ciprofloxacin	7	47 (100)
Erythromycin	15 (31.9)	32 (68.1)
Gentamycin	12 (25.5)	35 (74.5)
Methicillin	18 (38.3)	29 (61.7)
Oxacillin	22 (46.8)	25 (53.2)
Penicillin	47 (100)	0
Sulphamethoxazole+trimethoprim	3 (6.4)	44 (93.6)
Tetracycline	25 (53.2)	22 (46.8)
Vancomycin	20 (42.6)	27 (57.4)

DISCUSSION

The overall prevalence rate of coagulase positive staphylococci was 12.6% from both fresh and fermented milk in this study (Table 1). This appears low when compared to 37-43%, and 81.3%, reported by Umoh (1989) and Umoh *et al.* (1990) respectively in studies carried out in the same study areas [10,12]. The difference in the prevalence may be due to the fact that all the herds visited in the present study had used antibiotic (s) four weeks before the sample collection. Another reason could be that while the other study collected milk from nomadic Fulani settlers, the present study collected milk from herds supplying two peri-urban dairy groups (MILCOPAL, in Kaduna and NAPRI, in Zaria), who normally ensure proper hygienic measures during milk collection and processing. Also, the present study was conducted during the dry season (January to April), the period known to record low prevalence of organisms, and also the period during which the pH of milk tends to be low, which inhibits the growth of most organisms [12]. However, the results are in agreement with 15.9% reported by Umoh and Odoba [13] in street foods in Zaria, 15.9% reported by Zouharova and Rysanek [22] in Aydin, Turkey and 14.7% reported by Strastskova *et al.* [23] in Czech Republic in bulk tank milk respectively.

The presence of coagulase positive staphylococci in milk can be accounted for by secondary contamination from skin, mammary gland and the nasal cavity of milk processors, or alternatively, to unsatisfactory conditions of the environment [25]. Furthermore, inadequate handling of milk up to the

moment of pasteurization, allows for the production of thermostable toxins, which resist temperatures as high as 100°C for 30 minutes [26].

The isolation of the coagulase positive staphylococci from the different types of milk samples showed that bulk milk has the highest occurrence of 50 %, raw milk recorded 12 % while pasteurized milk and 'kindirmo' (locally fermented milk) recorded 5.9 % and 5.0 % respectively. No staphylococci was isolated in yogurt samples examined (Table 2). The higher detection rate of the staphylococci in the bulk milk samples compared to raw milk was not agreement with the report Zouharrova and Rysannek [23] who in their study showed that substantial mixing of contaminated milk substantially reduced the likelihood of staphylococci detection [23]. The decrease in the occurrence of coagulase positive staphylococci from raw milk to the final products (yogurt and 'kindirmo)', indicates that pasteurization and fermentation eliminated most of the organisms. This was evident by the low occurrence of staphylococci in the 'kindirmo' samples examined with its total absence in yogurt. This has also been documented by several studies that fermented foods are not good media for staphylococcal species growth [10].

Table 3. Multiple antimicrobial resistance profile of *S. aureus* isolated from fresh and fermented milk in Kaduna and Zaria, Nigeria.

Antibiotics	No. of isolates	% of the isolate
P	10	21.3
P, AMC, CN	1	2.1
P, AMC, MET	2	4.3
P, C, OX, CIP, AMC	1	2.1
P, E, OX, AMC, CN, MET	1	2.1
P, MET	3	6.4
P, SXT, OX, VA, AMC, MET	1	2.1
P, VA	1	2.1
P, VA, CN	1	2.1
P, VA, MET	1	2.1
TE, P	2	4.3
TE, P, AK, VA, AMC, CN	1	2.1
TE, P, E, AK	1	2.1
TE, P, E, OX	2	4.3
TE, P, E, OX, AMC	1	2.1
TE, P, E, OX, MET	2	4.3
TE, P, E, OX, VA	1	2.1
TE, P, E, OX, VA, AMC	2	4.3
TE, P, E, OX, VA, AMC, CN	3	6.4
TE, P, E, OX, VA, AMC, CN, MET	1	2.1
TE, P, E, OX, VA, MET	1	2.1
TE, P, E, SXT, OX, VA, AMC	1	2.1
TE, P, OX, VA, AMC, CN, MET	1	2.1
TE, P, OX, VA, AMC, MET	1	2.1
TE, P, SXT, C, MET	1	2.1
TE, P, SXT, OX, VA, AMC	1	2.1
TE, P, VA, AMC, CN, MET	1	2.1
TE, P, VA, MET	1	2.1
TE, P, OX, MET	1	2.1
TOTAL	**47**	**100.0**

The coagulase positive staphylococci isolates from this study were resistant to most of the antibiotics tested except ciprofloxacin with resistance to amikacin and chloramphenicol being very low. This indicates how dangerous these isolates could be if transmitted to humans. This implies that ciprofloxacin, amikacin and chloramphenicol could be reliably effective in the treatment of coagulase positive staphylococci infections in the study area. The antibiotic resistance profile of coagulase positive staphylococci isolated from ready-to-eat foods and cow milk have been documented in Nigeria [9, 13].
The resistance obtained by Kwaga and Adesiyun were erythromycin 0%, penicillin 47.1%, chloramphenicol 5.9%, ampicillin 33.3%, tetracycline 11.8% and streptomycin 3%. Those obtained by Umoh *et al.* (1989) were sulphafurazone 33.3%, penicillin 31.2%, ampicillin 26.4%, tetracycline 7.4% streptomycin 6.9% and erythromycin 5.2%. The findings from this study show that the current situation is significantly different, particularly with regards to penicillin (100%), erythromycin (31.9%) and tetracycline (31.9%), and most surprisingly methicillin (38.3%) and vancomycin (42.6%) (Table 3).

The disparity between the studies may be due to high rate of antibiotic abuse in both humans and animals, the differences in the concentration of the antibiotics used and sources of the isolates. The findings in this study are similar to those of Umoh and Odoba [13] who observed that more than 50% of the coagulase positive *S. aureus* were resistant to the common antimicrobial drugs used in the treatment of staphylococcal wound infections. The fact the 34 (73.4 %) of the coagulase positive staphylococci were resistant to 3 or more antibiotics indicate the isolates were implicate. The common multiple resistance patterns are tetracycline-penicillin, tetracycline-penicillin-erythromycin and tetracycline-penicillin-erythromycin-oxacillin-vancomycin (Table 4).

ACKNOWLEDGEMENTS
We thank Mallam Yahuza Suleiman Maitala of the Bacterial Zoonoses Laboratory, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria for technical assistance, and the management and staff of MILCOPAL Dairy Plant Kaduna and National Animal Production Research Institute (NAPRI), Zaria, for assisting during sample collections.

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