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Full Length Research Paper

Isolation, identification of *Staphylococcus aureus* from bovine milk and its antibiotics susceptibility

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The isolation, identification of *Staphylococcus aureus* from bovine milk and its antibiotic susceptibility was studied. One milliliter of each freshly drawn milk sample was inoculated into 9 ml of sterile peptone water and incubated overnight. A loop full of peptone water broth was streaked on blood agar base enriched with sheep blood using the quadrant streaking method for each quarter. Blood agar plates were incubated aerobically at 37°C for 24 h. *S. aureus* was identified by the tube coagulase test. Antimicrobial sensitivity test was conducted on *S. aureus* isolates using 18 antibiotics. From the study 43.75% had subclinical mastitis, while none was tested positive for clinical mastitis. None of the *S. aureus* isolates was fully sensitive to all the agents; 3 (42.86%) isolates were resistant to 10 agents, followed by 2 (28.57%) isolates which were resistant to 4 agents.

Key words: Milk, antibiotics, *Staphylococcus aureus*, mastitis, cow.

INTRODUCTION

Milk is a completely balanced diet with the right amount of carbohydrate, protein, fats, vitamins and minerals. Bacteria of sorts thrive in milk and as a result reduce its quality. The presence of pathogenic bacteria in milk is of immense public health significance. The hands of unhygienic milk handlers, the housing environments and instruments, and the cow itself are possible sources of milk contamination by pathogenic bacteria.

Staphylococcus aureus which is one of the causes of mastitis in cows could have its source from milk handlers since most humans carry the organisms in their nostrils. This is the most common type of mastitis and has great economic importance to dairy farmers (Abera et al., 2010). Mastitis in cattle caused by *S. aureus* can either be subclinical or clinical. According Bachaya et al. (2011), sub-clinical mastitis is of global importance in the dairy industry. It shows no noticeable alterations in the appearance of the milk or the udder, but there is decrease in milk production. The symptoms of clinical mastitis include swelling, hardness, redness, heat, and pain.

It is very important to implement application of an antibiotic susceptibility test prior to the use of antibiotics in both treatment and prevention of intra-mammary infections. This is because the presence of *S. aureus* or any pathogenic bacteria in milk can be a transmission pathway to humans. It becomes more dangerous when the pathogen develops resistance to antibacterial agents. Antibiotics resistance has become a very important public health issue globally (Abebe et al., 2013).

The aim of this study is to isolate and identify *S. aureus* from bovine milk and its antibiotics susceptibility.

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Table 1. *S. aureus* isolated from clinical and subclinical cases of mastitic cows.

Forms of mastitis	No examined	No of *S. aureus* isolated	Prevalence (%)
Clinical	16	0	0
Subclinical	16	7	43.75
Total	32	7	21.86

Table 2. Prevalence of *S. aureus* based on risk factors associated with subclinical mastitis.

Risk factor	Total No	No (%) positive	OR and 95% CI	P-value
Age				
0-3	9	3 (33.33)	1	0.614
4-6	7	4 (57.1)	0.39(0.03,4.23)	
Lactation stage				
0-3	5	5 (100)	1	
4-6	11	2 (18.18)	Inf(1.925,inf)	0.005

Key: OR – odds ratio, **CI** – Confidence interval.

MATERIALS AND METHODS

Sample collection

The macroscopic examination of the udders of the cows at the livestock farm of Federal College of Animal Health and Production Technology, Vom Nigeria, was done according to Sharma and Brinty (2014). This was done by applying gentle pressure with fingers on the udders for the presence of swelling, hardness, redness, heat, and pain. Moreover, the physical characteristic of the milk from each quarter was checked for any alterations. Before the milk samples were collected, each quarter was washed with tap water and dried. The teats were swabbed one after the other with cotton soaked in 70% ethanol. 10 ml of milk was then collected aseptically from the udders into sterile universal bottles after discarding the first three milking streams. Two replicates were carried out for each quarter. Samples from each quarter were transported on ice to Microbiology Laboratory of the Federal College of Animal Health and Production Technology, Vom Nigeria, where standard bacteriological assays followed.

Isolation and identification of bacteria

One milliliter of each sample was inoculated into 9 ml of sterile peptone water and incubated overnight. A loop full of peptone water broth was streaked on blood agar base enriched with sheep blood using the quadrant streaking method for each quarter. Blood agar plates were incubated aerobically at 37°C for 24 h. The plates were then examined for gross colony morphology, pigmentation and haemolytic characteristics at 24 - 48hrs. Presumptive colonies of *S. aureus* was selected and sub cultured on nutrient agar and incubated aerobically at 37°C for 24 to 48 h (Abera et al., 2010). After this, bacteria were identified by their Gram reaction, morphology, catalase test and coagulase test. *S. aureus* was identified by the tube coagulase test. Samples were considered positive for *S. aureus* when a colony was identified as *S. aureus.*

Antimicrobial resistance pattern test

Antimicrobial sensitivity test was conducted on *S. aureus* isolates. 18 antimicrobials were tested on the isolates (Cheesbrough, 2006). The following antimicrobial disks with their corresponding concentration were used: Pefloxacin (30 µg), amoxicillin (30 µg), gentamycin (10 µg), ampiclox (30 µg), zinacef (20 µg), rocephin (25 µg), ciprofloxacin (10 µg), chloramphenicol(30 µg), streptomycin (30 µg), septrin (30 µg), erythromycin (10 µg), norfloxacin (10 µg), levofloxacin(30 µg), ciproflox (10 µg), nalidixic acid (30 µg), amoxil (30 µg), tarivid (10 µg) and rifampicin (10 µg). The zones of inhibition were reported as the diameter of the clear zone surrounding the individual disk. Based on this, the isolates were defined as resistant, intermediate and sensitive.

Statistical analysis

The data obtained from this research work was analyzed using Chi-Square statistical method on R commander.

RESULTS

Of the total 16 lactating cows examined during the study period, 7 (43.75%) had subclinical mastitis, while none was tested positive for clinical mastitis (Table 1). From Table 2, it can be observed that there is no association between the prevalence of *S. aureus* and age below 6 years having an OR of 0.39 and 95% CI of 0.03, 4.23. There was also no association between the prevalence of *S. aureus* and lactation stage.

Considering age as a risk factor, using Chi-squared test of independence and a P-value of 5% significant level, $\chi^2 = 0.907$, df = 1, P-value = 0.3409, we accept the alternative hypothesis that there is a relation between age and the prevalence of *S. aureus* isolated from subclinical mastitic milk samples.

With regards to lactation stage as a risk factor and using Chi-squared test of independence and a P-value of 5% significant level, $\chi^2 = 9.3506$, df = 1, P-value = 0.0022,

Table 3. Resistance of *S. aureus* isolates to different antibiotics.

Antimicrobials	Resistant no (%)	Intermediate no (%)	Sensitive no (%)
Pefloxacin	3 (42.9)	3 (42.9)	1 (14.3)
Gentamycin	3 (42.9)	2 (28.6)	2 (28.6)
Ampiclox	7 (100)	0 (0)	0 (0)
Zinacef	6 (85.7)	1 (14.3)	0 (0)
Amoxacillin	7 (100)	0 (0)	0 (0)
Rocephin	7 (100)	0 (0)	0 (0)
Ciprofloxacin	3 (42.9)	1 (14.3)	3 (42.9)
Streptomycin	4 (57.1)	0 (0)	3 (42.9)
Septrin	6 (85.7)	0 (0)	1 (14.3)
Erythromycin	6 (85.7)	0 (0)	1 (14.3)
Levofloxacin	1 (14.3)	0 (0)	6 (85.7)
Amoxil	4 (57.1)	0 (0)	3 (42.9)
Chloramphenicol	1 (14.3)	4 (57.1)	2 (28.6)
Ciproflox	6 (85.7)	0 (0)	1 (14.3)
Nalidixic acid	5 (71.4)	1 (14.3)	1 (14.3)
Rifampicin	4 (57.1)	1 (14.3)	2 (28.6)
Tarivid	1 (14.3)	1 (14.3)	5 (71.4)
Norfloxacin	1 (14.3)	2 (28.6)	4 (57.1)

Table 4. Prevalence of multi-drug resistance of *S. aureus* isolates.

Parameter	Frequency of multi-drug resistance	
	Number	Percentage
Susceptible to all agents	0	0
Resistant to 1 agent	0	0
Resistant to 2 agents	0	0
Resistant to 3 agents	1	14.29
Resistant to 4 agents	2	28.57
Resistant to 5 agents	0	0
Resistant to 6 agents	0	0
Resistant to 7 agents	0	0
Resistant to 8 agents	1	14.28
Resistant to 9 agents	0	0
Resistant to 10 agents	3	42.86

we accept the null hypothesis that there is no relationship between lactation stage and the prevalence of *S. aureus* isolated from subclinical mastitic milk samples. From a total of 7 isolates of *S. aureus* obtained from the study, antimicrobial susceptibility tests were performed on 7 isolates. From Table 3, it will be observed that *S. aureus* was found to be highly sensitive to levofloxacin (85.7%), followed by tarivid (71.4%) and Norfloxacin (57.1%). However these isolates were highly resistant to amoxicillin (100%), ampiclox (100%) and rocephin (100%) followed by septrin (85.7%), erythromycin (85.7%) and ciproflox (85.7%).

Based on the prevalence of multi-drug resistance of *S. aureus* isolates shown in Table 4, none of the isolates was fully sensitive to all the agents; 3 (42.86%) isolates were resistant to 10 agents, followed by 2 (28.57%) isolates which were resistant to 4 agents.

DISCUSSION

The result obtained from this research indicates the occurrence rate of subclinical mastitis among the 16 lactating cows was 43.75% (7 cows) which is in line with

some earlier reports of 57.7% in cows in Maiduguri by Bamayi and Aniesona (2013). This report is higher than the report of Suleiman (2012) with a prevalence of 30.9% in subclinical mastitis and that of Ameh et al. (1999) with a prevalence of 31% from settled herds in Zaria.

In most countries and irrespective of the cause, the prevalence of mastitis is about 50% in cows. The infection rate in cows was close to the findings of Abdelrahim et al. (1990), who found a prevalence of 45.8% in Sudan. Of the total 16 lactating cows examined during the study period, 7 (43.75%) had subclinical mastitis, while none was tested positive for clinical mastitis as shown in Table 1.

Table 2 shows the prevalence of *S. aureus* based on risk factors associated with subclinical mastitis in the lactating cows showed that there is a relation between age (P = 0.3409) and the prevalence of *S. aureus* isolated from subclinical mastitic milk samples. Also, with regards to lactation stage as a risk factor, it was showed that there is no relationship between lactation stage (0.0022) and the prevalence of *S. aureus* isolated. From a total of 7 isolates of *S. aureus* obtained from the study, antimicrobial susceptibility tests were performed on 7 isolates. In this study *S. aureus* were found to be highly sensitive to levofloxacin (85.7%), followed by tarivid (71.4%) and norfloxacin (57.1%). However, these isolates were highly resistant to amoxicillin (100%), ampiclox (100%) and rocephin (100%) followed by septrin (85.7%), erythromycin (85.7%) and ciproflox (85.7%). The antimicrobial resistance profiles are shown in Table 3.

Based on the prevalence of multi-drug resistance of *S. aureus* isolates shown in table 4, none of the parameter is fully sensitive but highly resistant to 10 agents haven number 3 (42.86%), followed by 4 agents with number 2 (28.57%), 1 agents with number 1 (14.28%) and 8 agents haven number 1 (14.28%) in total of 100%.

Conclusion

The prevalence of *S. aureus* can most likely be attributed to the wide distribution of the organism inside mammary glands and on the skin of teats and udders. *S. aureus* adapts very well in the udder and establishes chronic and subclinical infections. From there it is shed into the milk, which serves as a source of infection for healthy cows during the milking process. Of the 16 lactating cows examined, 7 were positive for subclinical mastitis, which may be an indication of a future mastitis problem at the College farm. The antimicrobial susceptibility tests carried out in this study indicated the existence of resistant strains of *S. aureus.*

Conflict of Interest

The authors have not declared any conflict of interest.

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