

## Bacteriological Investigation of Bovine Clinical Mastitis in Two Selected Farms in Ibadan, Oyo State of Nigeria

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### ABSTRACT

Bacteriological examination was performed on 60 and 40 milk samples of clinical mastitic udders from Akinyele and Iyana Offa farms, Ibadan, respectively. A total of 251 bacterial isolates belonging to 5 bacteriological genera were recovered from the milk of the mastitic cows examined. Approximately 70% and 76% of the Staphylococci isolated from the milk of mastitic cows in Akinyele and Iyana Offa farms, respectively, were coagulase positive. *Staphylococcus aureus* was the most common pathogen in this study with an incidence of 39.0% while the coagulase-negative *Staphylococcus* species had an incidence of 3.6%. Other bacteria isolated include *Streptococcus agalactiae* and *Pseudomonas aeruginosa*, each with an incidence of 7.6%, while *Streptococcus uberis* showed an incidence of 18.7%. *Escherichia coli* and *Klebsiella pneumoniae* showed an incidence of 11.2% and 12.4%, respectively. The bacteria encountered in this investigation are among the pathogens that have been incriminated as contaminants of fresh milk meant for human consumption and are therefore of public health importance.

**Key words:** bacteria, cows, mastitis, staphylococci

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### INTRODUCTION

In tropical countries like Nigeria with predominance of *Bos indicus* (zebu) cattle, the average annual milk yield per milking cow is generally poor due to poor genetic potential, adverse climatic conditions and problems associated with mastitis. Mastitis is a very costly disease resulting in severe economic losses to the dairy industry and is appreciated by growing population of dairy farmers (Ojo and Falade, 1974; MacDonald and Low, 1985). Economic losses due to mastitis are in terms of reduced milk yield or milk quality, early culling of severely affected animals, expensive antibiotic treatment and veterinary services and losses of the young ones (Sordielli *et al.*, 2000; Leitner *et al.*, 2001; Brouillette *et al.*, 2003).

Although several pathogens can cause mastitis, *Staphylococcus aureus* and/or

*Streptococcus* species are the major bacterial agents of clinical and subclinical mastitis in most parts of the world (Ojo and Falade, 1974; Tollersrud *et al.*, 2000). *Staphylococcus aureus* constitutes the normal flora of the anterior nares and skin of man and animals (Kloss *et al.*, 1976; Ajuwape and Akinyede, 2001; Ajuwape and Aregbesola, 2002) and it has been demonstrated that cross infection of *Staphylococcus aureus* occurs between human and animals due to close contact (Adesiyun and Usman, 1983; Swartz *et al.*, 1985), therefore this organism constitutes a hazard to food hygiene and/or zoonotic threats. However, *Escherichia coli* is ubiquitous in cow's environment that is contaminated with feces (Watts, 1989; Jones, 1990).

In the Southwestern part of Nigeria a bacteriological study on the incidence of clinical mastitis in cows incriminated *Staphylococcus aureus* (34.5%), Streptococci (17.2%), *Pseudomonas aeruginosa* (6.9%), Coliform (20.6%); *Klebsiella aerogenes* (3.4%) and other

\*FOR CORRESPONDENCE

bacteria (6.9%) (Ojo and Falade, 1974). Recently, in a similar study in goats in the same region, Ajuwape *et al.* (2005) documented *Staphylococcus epidermidis* (50.9%), and *Escherichia coli* (15.1%), while *Streptococcus* species and *Bacillus cereus* had an incidence of 9.4% and 7.5%, respectively. *Mannheimia (P.) haemolytica*, *Corynebacterium* species and *Klebsiella pneumoniae* each showed an incidence of 5.7%. These findings indicate that the etiologic agents of mastitis keep changing hence, there is a need for regular monitoring for public health and economic reasons.

Therefore, this current paper reports the incidence of clinical mastitis in dairy herds located in Ibadan, Oyo State of Nigeria.

#### MATERIALS AND METHODS

In this cross-sectional study, bacteriological examination was performed on 60 and 40 milk samples of clinical mastitic udders from Akinyele and Iyana Offa dairy herds, respectively. Sampling started in July 2002 and continued until late March 2003. The animals involved in this investigation were White Fulani and Sokoto Gudali breeds managed under semi-intensive system. About 5 ml of milk from each clinical mastitic udder or quarter was aseptically collected into a sterile universal bottle before antibiotic treatment was instituted. The first squirts of milk were discarded as they may contain bacteria from within the streak canal. Samples were transported with ice (Coleman® Flask) to the Department of Veterinary Microbiology and Parasitology, University of Ibadan, Ibadan where they were examined bacteriologically.

The mastitic cow milk samples were inoculated onto 7% human blood agar (Oxoid Columbia blood agar®) and MacConkey agar No. 2 (Oxoid CM 109®) plates and were incubated aerobically at 37°C for 24-72 hr. Plates were examined for bacterial growth daily. Hemolysis and pigmentation were scored after 24 hr and isolates were examined for morphological, staining, cultural and biochemical characteristics according to standard methods (Cruickshank *et al.*, 1975; Barrow and Feltham, 1993). Colonies yielding

Gram-positive cocci in clusters which are catalase positive and oxidase negative, were subjected to biochemical test: the acid production of the isolates from carbohydrates was tested by agar plate method described by Cruickshank *et al.* (1975). The medium consists of 0.1% ammonium dihydrogen phosphate, 0.02% potassium chloride, 0.02% magnesium sulphate, 0.004% bromocresol purple and 1.5% agar in distilled water. Ninety-five milliliters of the medium was autoclaved at 121°C for 15 minutes and 5 ml of 10% sterile solution of each carbohydrate was added. The carbohydrates used include glucose, maltose, mannitol, lactose, sucrose, xylose, dulcitol, sorbitol and trehalose. Up to 10 isolates were spot-inoculated onto each plate and incubated at 37°C for 24-48 hr. The urease production was tested for each isolate on Christensen urea agar as described by Cruickshank *et al.* (1975).

The Staphylococci were subjected to coagulase test by slide and tube methods described by Langlois *et al.* (1990) using Oxford *Staphylococcus* (NT 6571) and sterile human plasma in broth as positive control. Slide coagulase was done by adding a drop of plasma to a drop of saline and mixing homogeneously; a positive result is seen as clumping within 15 seconds. Tube coagulase was done by making a 1:10 dilution of plasma then 0.5 ml of diluted plasma was added to 0.5 ml nutrient broth culture and incubated at 37°C. The mixture was inspected at 30-min intervals for clot formation. Any tube that did not form a clot at 24 hr was regarded as negative.

Other isolates were examined for their morphological, staining, cultural and biochemical characteristics according to standard methods described by Cruickshank *et al.* (1975) and Barrow and Feltham (1993).

#### RESULTS

A total of 251 bacterial isolates were recovered from the milk of 100 mastitic cows examined. On the basis of frequency of isolation *Staphylococcus aureus* (39.0%) was the most encountered organism in this study while the coagulase-negative staphylococci were the least common pathogen with an incidence of 3.6%.

Majority of the Staphylococci isolated from both farms were coagulase positive with an incidence of 70% and 76% from Akinyele and Iyan Offa farms, respectively. The biochemical activities of these Staphylococci are shown in Table 1.

*Streptococcus uberis* with an incidence of 18.7% was the second most common bacterium in this study, followed by *Klebsiella pneumoniae* at 12.4%. Furthermore, *Pseudomonas aeruginosa* and *Streptococcus agalactiae* each had an incidence of 7.6%. The incidence of *Escherichia coli* in the current investigation was 11.2% (Table 2).

### DISCUSSION

The highest incidence (39%) recorded for *Staphylococcus aureus* in cows with clinical mastitis indicates the predominance of this organism in cows in the area studied. This organism has been reported to be an important etiological agent of subclinical and clinical mastitis (Ojo and Falade, 1974; Tollersrud *et al.*, 2000). Hand-milking might have contributed to a cross infection of the strains of this organism between the milkers and the lactating cows as earlier documented by Garcia *et al.* (1990) who incriminated human *S. aureus* biotypes in bovine mastitis. Interestingly, the *S. aureus* in this investigation coagulated human plasma. The findings of the study was contrary to that of Lafi *et al.* (1994) wherein there was a predominance of coagulase negative *Staphylococcus* spp. on both clinical and subclinical mastitis in Jordanian cattle. Recently from a similar goat mastitis investigation, Ajuwape *et al.* (2005) recorded an incidence of 50.9% for coagulase-negative staphylococci, which were the predominant bacteria, whereas coagulase-negative staphylococci were previously regarded as non-pathogenic. Furthermore, coagulase-negative staphylococci have been reported to produce virulence factors being increasingly associated with clinical infections (Nobel, 1992). The above findings suggest that environmental bacteria apart from the hand-milkers or milking machine contribute to the type of organisms isolated from mastitis cases.

*S. aureus* remains one of the major pathogenic agents causing mastitis worldwide

Table 1. Biochemical characteristics of Staphylococci isolated from mastitic cows.

	Location		Total
	Iyana-Offa	Akinyele	
<b>Coagulase</b>			
<b>Slide</b>			
No. +	28	70	98
%	75.7	100	91.6
No. -	9	0	9
%	24.3	0	8.4
<b>Tube</b>			
No. +	28	70	98
%	75.7	100	91.6
No. -	9	0	9
%	24.3	0	8.4
<b>Biochemical reactions</b>			
<b>Glucose</b>			
No. +	37	70	107
%	100	100	100
No. -	0	0	0
%	0	0	0
<b>Maltose</b>			
No. +	34	63	97
%	91.9	90.0	90.7
No. -	3	7	10
%	8.1	10.0	9.4
<b>Mannitol</b>			
No. +	28	70	107
%	75.7	100	100
No. -	0	0	0
%	0	0	0
<b>Lactose</b>			
No. +	28	70	107
%	75.7	100	100
No. -	0	0	0
%	0	0	0
<b>Sucrose</b>			
No. +	28	70	107
%	75.7	100	100
No. -	0	0	0
%	0	0	0
<b>Xylose</b>			
No. +	26	32	58
%	70.3	45.7	54.2
No. -	11	38	49
%	29.7	54.3	45.8
<b>Dulcitol</b>			
No. +	37	70	107
%	100	100	100
No. -	0	0	0
%	0	0	0
<b>Sorbitol</b>			
No. +	37	70	107
%	100	100	100
No. -	0	0	0
%	0	0	0
<b>Trehalose</b>			
No. +	34	70	104
%	91.9	100	97.2
No. -	3	0	3
%	8.1	0	2.8
<b>Urease</b>			
No. +	32	70	102
%	86.5	100	95.3
No. -	5	0	5
%	13.5	0	4.7

Key: +: positive, -: negative

Table 2. Bacteria isolated from mastitic cows in Ibadan, Nigeria.

Isolate	Frequency*	Incidence (%)
<i>Staphylococcus aureus</i>	98	39.0
<i>Streptococcus uberis</i>	47	18.7
<i>Klebsiella pneumoniae</i>	31	12.4
<i>Escherichia coli</i>	28	11.2
<i>Streptococcus agalactiae</i>	19	7.6
<i>Pseudomonas aeruginosa</i>	19	7.6
Coagulase-negative <i>Staphylococcus</i> species	9	3.6

Total no. of isolates: 251

\*Some isolates occurred as multiple infections in mastitic cows.

and therefore necessitates the use of antibiotics in dairy herds. MASTIVAC I (Patent no IL122829, PTC/IL98/00627, AU746285, USA09/582692), a new vaccine, under field conditions and controlled experiments, was found to be effective against infection with virulent field strains of *S. aureus* (Leitner *et al.*, 2004). Such vaccine could be employed to vaccinate dairy cows to reduce the incidence of *S. aureus* infection. This is against the backdrop of increasing antibiotic resistance shown by the organism and the desire to minimize antibiotic residues in milk.

The high incidence of *Streptococcus uberis*, the second most common bacteria encountered in this study, and *Streptococcus agalactiae*, is in agreement with reports of increasing incidence of *Streptococcus uberis* mastitis throughout the world (Hogan and Smith, 1997; Tollersrud *et al.*, 2000). Their presence may contribute to the particular mastitis case being contagious (Garcia, 2004). The coliforms (*Escherichia coli* and *Klebsiella pneumoniae*) encountered in this study were similarly observed by Watts (1989), Jones (1990), Hogan and Smith (2003) and Garcia (2004), that they are most common environmental bacteria frequently incriminated in bovine mastitis. In this investigation the incidence of *Pseudomonas aeruginosa* (6.9%) is slightly higher than the value reported by Ojo and Falade (1974).

Once inside the mammary gland, these organisms multiply and damage the cells of the alveoli and their supporting structures, resulting in their progressive involution, reduced milk production potential and subsequent economic losses. The bacteria encountered in this investigation are among those that have been incriminated as sources of contamination of fresh organic milk meant

for human consumption and are therefore of public health importance.

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