

Aerobic Bacteria Pathogens Associated with Caprine Mastitis in Nsukka Area of Enugu State

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Abstract

This study ascertained the aerobic bacteria associated with cases of clinical and subclinical caprine mastitis. A total of 58 lactating West African dwarf does were used for this investigation. These lactating does had signs of clinical and sub-clinical mastitis. These samples were collected from Nsukka town, Nsukka abattoir, Orba Market, Obukpa Market and Obollo - Afor market, all in Enugu State, Nigeria. Clinical mastitis was detected by gross signs of udder infection by physical examination of abnormal milk, whereas subclinical mastitis was recognized using California Mastitis test. This study showed that 39 (67.24%) of 58 goats were positive for mastitis on California Mastitis Test. Clinical and subclinical cases of caprine mastitis, *Staphylococcus aureus* was the predominant pathogen 32 (55.17%). 14 (24.13%) of the isolates were positive for *Staphylococcus epidermidis*, 6 (10.34%) for *Streptococcus agalactiae*, 4 (6.89%) *E.coli* and 2 (3.44%) for *Klebsiella pneumoniae*. This work identified the bacterial species linked with caprine mastitis in Nsukka area of Enugu State.

Key words: Clinical mastitis, subclinical mastitis, aerobic bacteria, lactating, caprine.

Introduction

In Nigeria, one of the major limiting factors to the successful rearing of goats and sheep is disease, mastitis is a major one (Ugochukwu, 2008). The importance of goats as a source of meat and dairy products has been well discussed and documented by Haenlein (2004). Nigeria have an estimated goat population of 34.5 million (Lawal-Adebowale, 2012) but very little of this large population, is used for dairy purposes (Ugochukwu, 1983b). For this reason, little attention has been directed to study the incidence and proffer control solution for caprine mastitis.

Mastitis, an inflammatory reaction of the udder tissue to bacterial, chemical, thermal or mechanical injury is characterised majorly by physical, chemical and bacteriological changes in milk with attending necrotic, pathological and glandular structural changes in defined areas of

the udder (Togun *et al.*, 2003). Mastitis is one of the major diseases in veterinary medicine. The global economic losses due to mastitis is about \$35 billion annually. In addition to the huge direct and indirect economic losses, the presence of certain pathogens in the milk is a major threat to public health (Bedolla and Castañeda, 2003; Bilal *et al.*, 2004; Wolter *et al.* 2004; Ali *et al.*, 2010). Mastitis is a complex disease with multiple predisposing factors and various causative agents (Harmon *et al.*, 1990; Radostits *et al.*, 2000; Tollersud *et al.*, 2000; Bedolla, and Castañeda 2003; Bergioner *et al.*, 2003). It is a disease complex which is dependent on several factors such as aetiologic agents, management practices such as the use of milking machine, susceptibility to the disease and efficiency of the host defence mechanisms (Ugochukwu, 1983b). Clinical mastitis may be easy to detect but animals suffering from subclinical mastitis are very difficult to diagnose since there is a lack of reliable

diagnostic methods, especially at farm level (Leitner *et al.*, 2004). Early diagnosis of subclinical mastitis is vital

Because changes in udder tissue take place much earlier than they become apparent (Contreras, 2007). However, the diagnosis of subclinical mastitis in goats is not easy but direct bacteriological assay could be used (Maisi and Riipinen, 1988; Maisi, 1990; Fthenakis, 1995; Gonzalez-Rodriguez and Carmenes, 1996; Sanchez *et al.*, 2004).

Clinically, affected glands frequently suffer partial or complete damage and do not resume normal function (Fthenakis and Jones, 1990; Mørk *et al.*, 2007). Most of the cases of mastitis studies show serious bacteria aetiological involvement. Many bacterial strains has been implicated in the aetiology of caprine mastitis, some frequently isolated organisms includes: *Staphylococcus* spp, *Streptococcus* spp, *E.coli*, *Corynebacterium* spp. and *Nocardia* spp (Ugochukwu, 1983a; Ameh and Tari, 2000; Ajuwape *et al.* , 2005 Mørk *et al.*,2007). Some other bacterial species incriminated include *Pseudomonas aeruginosa*, *Mannheimia haemolytica*, *Klebsiella* spp, *Pasteurella* spp, *Listeria monocytogenes* and Fungi (Adetunji and Olaoye, 2012; Hawari *et al.*, 2014). Control measures for mastitis are important in order to reduce economic losses and decrease the risk of diseases, being transmitted to humans via food (Cortimiglia *et al.*, 2015).

There has been little knowledge of the aetiology of this disease. Goat and sheep industries is gaining grounds in some part of Nigeria, coupled with the dearth of information in available scientific literature on caprine mastitis in Nsukka area and the Southeastern part of Nigeria, necessitated this bacteriological investigation.

Materials and Methods

Study area

The study areas are Nsukka and Obukpa market in Nsukka Local Government Area, Orba and Obollo Afor market in Udenu Local Government Area, Enugu State, Nigeria. Nsukka is situated at latitude 6°51'24"N and longitude 7° 23'45"E, with a total Landmass of 17.52 sq. miles (45.38km²), an annual mean humidity of 13% and an elevation of 1,810ft (550m) above sea level. The geographical coordinates for Orba is 6°51'0"N, 7°27'0"E, while Obollo is located at 6°55'N 7°31'E.

Study animals

Animals used for this investigation were 58 lactating West African dwarf does with obvious clinical signs of clinical and sub-clinical mastitis.

Sample collection

Samples were collected from clinical and sub-clinical cases of mastitis. The milk samples were collected under complete aseptic conditions.

Diagnosis of Clinical and sub-clinical mastitis

Clinical mastitis was detected by gross signs of udder infection during physical examination of abnormal milk, while subclinical mastitis was recognized using California Mastitis test (CMT) following standard procedures described by Assefa, *et al.* , (2006). Milk samples of the goats with clinical and subclinical mastitis were subjected for isolation and identification of bacterial pathogens.

Isolation and identification of bacterial agents

Samples were kept in the refrigerator at 4°C within a maximum of 12h after sampling before examination. The samples were centrifuged at 1500 rpm for 30min as recommended by Yousof – Beighi *et al.* (2005). The sediments were cultured in Blood agar, MacConkey agar and Nutrient agar plates. The inoculated plates were incubated under aerobe conditions at 37°C and colonial growth was checked after 24 h. The colonial morphology and type of haemolysis were recorded. After Gram staining, the suspected pure colonies of gram positive were also identified by culturing in Mannitol salt agar, Catalase Test and Tube Coagulase Test using standard procedures as described by Chessbrough (2000). The Gram negative rods were subjected to Giemsa staining, indole, sugar utilization and urease tests as well as reaction on eosin methylene blue agar and Triple sugar iron agar (TSI agar) according to standard procedures described by Chessbrough (2000). Identification of the bacterial agents from the pure culture were based on colony characteristics, gram staining reaction, hemolysis pattern and biochemical test described by Chessbrough (2000).

Results and Discussion

Bacteriological findings

The distribution of aerobic bacteria isolated from secretions from the glands with clinical and sub-clinical mastitis is shown in Figure 1. *Staphylococcus aureus* was the predominant pathogen and was 32 (55.17%) of the samples from the mastitic glands. 14 (24.13%) of the isolates were *Staphylococcus epidermidis*, 6 (10.34%) of the isolates were *Streptococcus agalactiae*, 4 (6.89%) of the isolates were positive for *E. coli* and 2 (3.44%) of the isolates were positive for *Klebsiella pneumoniae* as shown in Figure 2. Table 1 shows biochemical tests and physiological test: Catalase, Tube coagulase, oxidase, indole, urease tests and sugar utilization tests.



Figure 1: Mastitic teat of West African dwarf Doe arrows showing the inflamed teat

California Mastitis test results

In this test, out of 58 lactating does, 39 (67.24%) were positive for mastitis on California Mastitis

Test (CMT), this is shown in Table 2. 28 (71.79%) of the West African Dwarf goats were positive for mastitis on CMT, while 11(28.20%) of the Kano Brown goats were positive for mastitis on CMT. A mastitic teat from the two examined breeds observed with mastitis is shown in Figures 2 and 3.



Bacteriological findings

The distribution of aerobic bacteria isolated from secretions from the glands with clinical and sub-clinical mastitis is shown in Figure 3. *Staphylococcus aureus* was the predominant pathogen and was 32 (55.17%) of the samples from the mastitic glands. 14 (24.13%) of the isolates were *Staphylococcus epidermidis*, 6 (10.34%) of the isolates were *Streptococcus agalactiae*, 4 (6.89%) of the isolates were positive for *E. coli* and 2 (3.44%) of the isolates were positive for *Klebsiella pneumoniae* as shown in Figure 2. Table 1 shows biochemical tests and physiological test: Catalase, Tube coagulase, oxidase, indole, urease tests and sugar utilization tests.

Table 1: Physiological tests and biochemical tests results for the aerobic bacteria isolated from secretions from the glands of lactating does with clinical and subclinical mastitis in Nsukka area

Aerobic bacteria isolate	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>S. agalactiae</i>	<i>E. coli</i>	<i>K. pneumoniae</i>
Tests					
Gram	+ve	+ve	+ve	-ve	-ve
Catalase	+ve	+ve	-ve	+ve	+ve
Coagulase	+/-ve	-ve	-ve	ND	ND
Oxidase	-ve	-ve	-ve	-ve	-ve
Indole	-ve	-ve	-ve	+ve	-ve
Urease	+ve	+ve	-ve	-ve	+ve
Motility	-ve	-ve	-ve	+ve	-ve
D-glucose	+ve	+ve	+ve	+ve	+ve
Sucrose	+ve	+ve	+ve	+ve	+ve
Lactose	+ve	+ve	-ve	+ve	+ve
Maltose	+ve	+ve	+ve	+ve	+ve
D-mannitol	+ve	-ve	-ve	+ve	+ve
Triple Sugar Iron Agar					
H ₂ S production	-ve	+ve	-ve	-ve	+ve
Gas production	-ve	+ve	-ve	+ve	+ve

*ND - Not done

Table 2: Breed distribution of lactating does with clinical and subclinical mastitis in Nsukka area

Breed	Number of affected lactating Does	Number CMT
+ve		
West African Dwarf	41	28
Kano brown	17	11
Total	58	39

Caprine mastitis is caused by bacteria of primary or secondary involvements. The udder could also be exposed to trauma from hard objects like

broken bottles and rusty metals in their scavenging environment, which make the goats susceptible to mastitis - causing organisms

(Togun *et al.*, 2003). Raw goat's milk can be a potential source of antibiotic resistant pathogens on animal, human, and environment. Microorganisms which contaminate raw milk may originate from the farm environment or goats which include aetiological agents responsible for clinical and subclinical mastitis (Virdis *et al.*, 2010).

In this study the bacterial isolates include *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus agalactiae*, *E. coli* and *Klebsiella pneumoniae* this agrees with the reports of Ugochukwu, (1983a); Ndegwa *et al.*, (2001); Sousa *et al.*, (2007); Ali *et al.* (2010); Islam *et al.* (2011); Hawari *et al.* (2014).

Out of the 58 isolates, *Staphylococcus aureus* had the highest isolation occurrence of 32 (55.17%). 9 (28.12%) out of the 32 *Staphylococcus aureus* isolates were Coagulase negative *Staphylococcus aureus*. Coagulase-negative staphylococci isolated in this study, although not a major pathogen of clinical mastitis in goats, has been shown to persist throughout the lactation and dry periods, irritating the gland and causing a decrease in production and even clinical mastitis (Ndegwa *et al.*, 2001).

Staphylococcus aureus has been incriminated in much suppurative conditions in man and animals (Greenwood *et al.*, 2002; Kitara *et al.*, 2011). Coagulase positive *Staphylococcus aureus* is known to be involved in mastitis in most species of domestic animals including goats, pigs, cattle and sheep (Radostiis *et al.*, 2000). It has also been isolated with the highest frequency in other works as reported by Mørk *et al.*, (2007), Ali *et al.*, (2010) and Islam *et al.*, (2011). The occurrence of a high number of coagulase positive *Staphylococcus aureus* suggest its involvement in the causation of caprine mastitis. *Staphylococcus epidermidis* isolated in this study is widely known to be harmless and the precise role of these bacteria in the pathogenesis of mastitis is not understood but has been associated with subclinical mastitis. It has been isolated by Contreras *et al.*, (2003) and Razi *et al.*, (2012).

Escherichia coli is a normal microflora of the gut. It is usually present in the faecal materials. Contamination of the udder by faecal materials could have led to the presence of the organism in the milk. Isolation of *E. coli* in this study agrees with the findings of Islam *et al.*, (2011), Hawari *et al.*, (2014) and Mbindyo *et al.* (2014).

Streptococcus agalactiae, a notorious agent for mastitis (Kateeke *et al.*, 2013). Castañeda *et al.*, (2013) and Hawari *et al.* (2014), reported isolation and identification of this organism from clinical cases of mastitis in their works on mastitis in caprines, bovines and ovines, in Northern Nigeria, Mexico and Al-Balqa province in Jordan, respectively.

Bacteria such as *Streptococcus dysgalactiae*, *Corynebacterium* spp., *Mannheimia haemolytica*, *Pasteurella multocida*, *Arcanobacterium pyogenes*, *Clostridium perfringens* which have been associated with chronic mastitis, that have been isolated by Mørk *et al.*, (2007), Ibrahim *et al.*, (2009), Ahmadi *et al.*, (2014) and Hawari *et al.*, (2014).

The differences in the Breed distribution of Mastitis, in which there was more cases in WAD goats compared to Kano Brown goat is due to the fact that WAD goats are the predominantly reared goat breed in Southeastern Nigeria.

In the present circumstances, goats and sheep industry is gaining considerable attention since 1983 as reported by Ugochukwu, 1983b. It is important as part of control measures to encourage indigenous farmers to report cases of mastitis to the nearest veterinary clinics.

In conclusion, this work identified the bacterial species involved in caprine mastitis in Nsukka area of Enugu State. It is believed that this investigation will stimulate research on other pathogens associated with caprine mastitis

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Conflict of Interest

There is no conflict of interest regarding the manuscript.

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