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https://doi.org/10.4314/nvj.v47i2.3 **ORIGINAL ARTICLE**

Bacteriological Evaluation of Pooled Gut Contents of Engorged *Hyalomma* Species Infesting Goats in Maiduguri, Borno State

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ABSTRACT

This study evaluated the bacteria from homogenized midgut of *Hyalomma* ticks of goats in Maiduguri, Nigeria using cultural isolation and biochemical characterization of homogenized migut of *Hyalomma* ticks. A total of eleven (11) isolates were obtained including *Staphylococcus aureus* with the highest frequency of 3(27.3%), *Enterococcus* species, *Proteus mirabilis* and *Bacillus* species with 2 (18.2%) each and *Escherichia coli* and *Corynebacterium* species with 1 (9.1%) each. Descriptive analysis of the bacterial isolates revealed *Escherichia coli* appearing greyish white with large, thick, moist, smooth, opaque or mucoid colonies on MacConkey agar, and as pinkish, rod or bacilli, gram negative, indole, nitrate reduction and MR-VP positive but oxidase negative. *Staphylococcus aureus* appeared fairly large golden yellow colonies on rich medium and round opaque and as purple violet colour, spherical or cocci in cluster, gram positive, catalase, glucose and coagulase positive but oxidase negative. *Proteus mirabilis* appeared swarming with a characteristic smell on MacConkey Agar, slender rods, gram negative, indole, citrate, lactose, sucrose, mannitol, urease positive. *Enterococcus* Species appeared mucoid on MacConkey Agar, in pairs or chain with rod shape, gram positive and lactic acid fermenters. *Bacillus* species appeared as circular ridges, smooth, moist, sticky and medium sized colonies, arranged in long chain, rod, gram positive, and catalase positive. *Corynebacterium* species appeared small, grayish colonies with granular appearance, mostly translucent but with opaque centers, convex with continuous borders, rod or bacilli and shows metachromatic granules when stained with methylene blue, gram positive, catalase positive some are oxidase negative. Further study is needed to understand the relationship between midgut bacterial community and tick–pathogen interaction

Keywords: Hyalomma, goats, gut content, bacteria, culture, characterization

INTRODUCTION

Ticks are vectors that are of significant importance in the transmission of various pathogens, including bacteria, viruses and protozoa, which cause diseases such as anaplasmosis, Lyme disease and tick-borne encephalitis (TBE) (Madison-Antenucci *et al*., 2020). Tick infestations result in economic losses from reduced productivity, veterinary costs and acaricide use (Pegram *et al*., 1993; Kasaija *et al*., 2021), the presence of ectoparasites in goat production has caused substantial losses in livestock production worldwide (Sonenshine,1991).

Hyalomma originated from Iran or Southern part of the former Soviet Union and spread into Asia, the Middle East, Southern Europe and Africa (Roberts *et al*., 2009). *Hyalomma* are large in size and do not have protective shields (indistinct festoons), but have eyes and banded legs. *Hyalomma* transmits species of pathogens Babesia, Anaplasma, Ehrlichia, Theileria and Trypanosomes, as well as, several viruses (Aktas *et al*., 2004; Aktas *et al*., 2009). Human-biting hard ticks are vectors for different types of infectious agents, they can carry different pathogens and cause important human diseases (Parola and Raoult, 2001). This paper presents a preliminary study of culturable bacterial pathogens from *Hyalomma* ticks recovered from goats sold or bought from Kasuwan Shanu in Maiduguri.

MATERIALS AND METHODS

Study Area

The study was conducted in Maiduguri metropolis, capital city of Borno State, North-East Nigeria at 11,856920 latitudes and 13,157120, longitude, at an elevation of 325m above the sea level. The climate of Maiduguri is characterized by a long period of dry season for the most part of the year (Hess *et al.,* 1995).

Collection of Ticks

Tick collection was done once every week for four weeks in February 2022, during visits to Kasuwan Shannu market, Maiduguri. During this period, ticks were carefully handpicked following the physical inspection of goats brought to the livestock market. Ticks were collected in pool of 10 making a total of forty ticks into well labeled universal containers.

Identification of Ticks

The ticks were processed in the laboratory of the Department of Veterinary Parasitology and Entomology Laboratory of the University of Maiduguri after collection from the individual goats and were placed separately into Petri dishes and examined under a dissecting microscope and identified using the classification keys of Soulsby (1982) and Fleck and Moody (1982).

Washing and Preparation of Tick Homogenates in the Laboratory

A 70% alcohol was used for washing the ticks and then rinse with normal saline in the laboratory. Sterile pestle and mortar were used for grinding the *Hyalomma* ticks to obtain the homogenate.

Inoculation of Tick Homogenates on Media

The media used for the purpose of this research were blood and the MacConkey agar respectively, after the tick sample is being

homogenized, it was then inoculated on a media, these are two different differential media used to cultivate microorganisms, the blood agar is enriched with blood nutrients, and it provides enough nutrients in which most microorganisms utilize for growth, while MacConkey agar is used to select gram-negative bacteria from non-fermenting bacteria respectively. Prepared blood agar plate was first used for inoculation of the homogenate of homogenized *Hyalomma* species using sterile wire loop where the suspected bacteria grow and reproduce. A prepared MacConkey agar plate was used to obtain a pure culture growth.

Incubation for Culture Growth

After being inoculated on the media, each plate was then kept in the incubator for eighteen (18) to twenty-four (24) hours and growth was observed. However, in most cases when no growth was obtained, the incubation period was extended for duration of forty-eight (48) hours so as to ensure full lap count and then results were taken from the media. However, if no growth was observed the result is reported as no growth.

Isolation of Colonies and Identification

Colonies of the suspected bacteria were isolated by streaking method over the exterior of the MacConkey agar plate, each of the isolated colonies were subjected to gram staining and microscopy for identification where more than one stain was used and the bacterial isolates were differentiated into two large groups of Gram negative and Gram positive based on cell wall constituents and coloring properties.

Biochemical Testing of Bacterial Isolates

Catalase test, Citrate test, Coagulase test, Carbohydrate fermentation test (sugar fermentation test), indole production test, Mannitol salt agar, Methyl red test/ Voges-Proskauer test (MR-VP broth), nitrate reduction test, oxidase test, TSI (Triple Sugar Iron) test and Urease test were the biochemical tests carried out in this study.

Catalase test: This test was carried out to identify organisms that produce the enzyme catalase. This enzyme detoxifies hydrogen peroxide by breaking it down into water and gas (Watson, R. M., 2019).

Citrate test: This test was carried out to determine the ability of the microbes to ferment citrate as sole carbon source (Watson, R. M., 2019).

Coagulase test: This test was carried out to determine the ability of microorganisms to produce coagulase which clots plasma and distinguish the pathogenic positive *Staphylococcus* from the non-pathogenic coagulase negative *Staphylococcus* (Watson, R. M., 2019).

Carbohydrate fermentation test (sugar fermentation test): This test was carried out to determine the ability of microorganisms to ferment carbohydrates with the production of an acid and/gas (Watson, R. M., 2019).

Indole production test: This test was done to determine the ability to microorganisms to degrade the amino acid tryptophan (Watson, R. M., 2019).

Methyl red test/ Voges-Proskauer test: This test was carried out to differentiate *Escherichia coli* and *E. aerogenes* and to determine the ability of microorganisms to

oxidize glucose with production and stabilization of high content of acid end product (Watson, R. M., 2019).

Nitrate reduction test: This test was carried out to determine the ability of some microbes to reduce nitrate to nitrites or beyond the nitrate stage (Watson, R. M., 2019).

Oxidase test: This test was done to identify microorganisms containing the enzyme cytochrome oxidase (important in the electron transport chain). It is commonly used to distinguish between oxidase negative *Enterobacteriaceae* and oxidase positive *Pseudomadaceace* (Watson, R. M., 2019).

TSI (triple sugar iron) test: This test was done to differentiate among and between the members of the *Enterobacteriaceace* family (Watson, R. M., 2019).

Urease test: This test was done to identify bacteria capable of hydrolyzing urea using the enzyme urease. It is commonly used to distinguish the genus *Proteus* from other enteric bacteria (Watson, R. M., 2019)

RESULTS

Table 1: Bacterial Isolates from Homogenized Midgut of *Hyalomma* Ticks

Bacteria	Frequency (%)
Escherichia coli	1(9.1)
Enterococcus species	2(18.2)
Proteus mirabilis	2(18.2)
Staphylococcus aureus	3(27.2)
Bacillus species	2(18.2)
Corynebacterium species	1(9.1)
Total Isolates	11(100%)

Table 2: Characterization of Bacterial Isolates from Homogenized Midgut of *Hyalomma* Ticks

Bacterial species	Appearance on media	Appearance on microscopy	Staining	Biochemical characteristics
Escherichia coli	Colonies are large, flat greyish white, moist, smooth, opaque or mucoid	Pink color, rod in shape, are bacilli with no specific arrangement.	Gram negative	Indole positive nitrate reduction test, oxidase negative MR-VP broth positive.
Staphylococcus Aureus	Fairly large golden yellow colonies on rich medium and round opaque	Purple based on colony property, the organisms are violet color while the shape appears spherical showing they are cocci and are arranged in clusters	Gram positive	Oxidase negative catalase producing glucose fermenters, coagulase positive on tube coagulase test
Proteus species	Swarming growth with a characteristic smell	Slender rods	Gram negative	Indole, citrate, lactose, maltose, sucrose,mannitol, urease positive
Enterococcus species	Mucoid colonies on MacConkey agar	Occurs in pairs or chain with rod shape	Gran positive	Lactic acid fermenters
Bacillus species	Circular ridges, smooth, moist sticky and medium sized colonies	Arranged in long chain, they are rod in shape	Gran positive	Catalase positive

Corynebacterium Species	Form small greyish colonies with a granular appearance, mostly translucent, but with opaque with continues borders	Rod-shaped bacilli with metachromatic granules when stained with methylene blue	Gram positive	Catalase positive some are oxidase negative

DISCUSSION

The culture of bacteria from homogenized midgut of *Hyalomma* ticks infesting goats in this study resulted the isolation of *Staphylococcus aureus*, *Enterococcus spp.*, *Proteus mirabilis*., *Bacillus spp*., *Escherichia coli* and *Corynebacterium spp.* The isolation of up to six different bacterial genera in the homogenized specimens from Hyalomma midgut was not unexpected, since previous microbiome studies have identified up to hundreds of bacterial genera associated with a single or pooled tick specimens (Khoo *et al*., 2016; Lado *et al*., 2018). Bacteria from the genera Staphylococcus, Bacillus, Enterococcus, Proteus, Escherichia and Corynebacteriumwere isolated in this study, were reported across many tick species (Andreotti *et al*., 2011; Clow *et al*., 2018; Ruiling *et al*., 2019), suggesting that these bacteria may have a role in the biology of the tick or the transmission of tick pathogens. Though these bacteria are not normally considered as tick pathogens. Staphylococcus found in this study agrees with the finding of Foster, 2012 who isolated Staphylococcus sp. in *Rhipicephalus microplus* ticks found on dairy cattle, these bacteria are innocuous commensals on livestock skin (Foster, 2012). It also agrees withAnbalagan *et al*., 2041 who isolated Staphylococcus spp. as the most common bacteria in the midgut of *Hyalomma anatolicum*. The isolation of high quantity of Bacillus genera, is in agreement with the study that found bacteria from the genus Bacillus being the most dominant genus isolated from Ixodes ticks in the United States (Martin & Schmidtmann, 1998).

The gut of ticks is an important organ in terms of blood digestion, absorption, excretion, pathogens migration, invasion and development (Li, *et. al.,* 2014). Ticks remain one of the most economically important parasites of domestic animals in tropical and subtropical countries. Ticks serve as reservoirs for bacteria and contain their bodies throughout their lives time (Sultana *et al.*, 2015). Ixodid ticks play an important role in the transmission of a variety of viral, bacterial and protozoan zoonoses and also harbor a wealth of micro-organisms. Tick do feed and carry bacteria from their environment. These bacteria can survive in ticks and some do replicate in them. Hard ticks feed on mammals for several days and

so have the opportunity to ingest microbes (Li, *et. al.,* 2014).

There is close association of ticks with bacteria with reports showing ticks as reservoirs of *Staphylococcus aureus* which predisposes livestock to abscess from septicemia, tick borne fever, tick borne typhus and complicated dermatophilosis. *Corynebacterium species* causes nasal, nasopharyngeal and tonsillar diphtheria often with marked oedema of the neck and toxaemia with cardiac and neurologic complications. *Proteus* causes urinary tract infection, abdominal and wound infection, as well as septicemia (Barandika *et. al*., 2008; Zaria *et al*., 2009 and 2011)

It has not been determined whether ticks have specific role in the perpetuation and spread of *Staphylococcus aureus* or whether their role is merely that of mechanical transmission. Biu *et. al*., (2015) isolated *Streptococcus*, *Corynebacterium* and *Proteus* species from *Hyalomma*, *Amblyomma* and *Rhipicephalus* ticks in Maiduguri. Which is similar to recent findings from this present work.

CONCLUSION

The identification of these bacterial species within ticks has several implications:

Disease Transmission: Ticks harboring pathogenic bacteria like E. coli, Staphylococcus aureus, Corynebacterium spp, Proteus spp and others found in this study could potentially transmit these pathogens to their hosts during feeding and cause infection.

The presence of diverse bacterial communities within ticks raises concerns about the potential for antibiotic resistance especially if these bacteria interact with other microbial populations in the environment or within hosts.

The microbial composition of ticks can aid in developing targeted strategies for tick control;

The findings of this study do show that in addition to common intracellular tick-borne pathogens such as the rickettsial agents, other extracellular bacterial pathogens (gut content of ticks) associated with human or animal infections could also be recovered, and this provide a preliminary information of the culturable bacterial species in ticks of livestock especially goats.

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