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Isolation and Identification of Pathogen Bacteria Associated with Farm Animals

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Abstract: Farm animals' meat contributes significantly to the daily protein intake of many individuals but can also be a source of foodborne illnesses especially under the conditions in which animals are handled, slaughtered, transported and sold. The emergence and re-emergence of diseases due to pathogenic bacteria are the key issue of the new pattern of food trades. A total of twenty-one samples (VIS, FSA, STS, MSA, URS, UDS, and PES) were collected from farm animals from Rugga settlement of Birnin Kebbi. Some of the samples were collected using sterile swab stick while urine and stool samples were collected in sterile containers and were taken immediately to Microbiology Laboratory Waziri Umaru federal Polytechnic. The samples were bacteriologically analyzed and the isolates were identified using biochemical tests such as (indole, coagulase, catalase, urease, gluctose, lactose, maltose, motility, Voges Proskauer and methyl red). Fourteen bacterial species were isolated and identified as pathogens from the sheep samples. They are Gram positive bacteria such as Streptococcus agalactiae and Staphylococcus aureus as well as Gram negative bacteria such as Escherichia coli and Shigella sp and Salmonella sp. The isolates were identified based on their reaction to biochemical tests. The findings revealed that sheep are potential vehicles for transmitting pathogenic bacteria and the presence of these microorganisms may lead to poisoning and can as well claim the live of the sheep from which they were isolated.

Keywords: Farm Animal, Foodborne Illness, Pathogenic Bacteria, Sheep

1. Introduction

Farm animal play an important role in the nutrition and income of people around the world. They serve as source of meat, milk, skin and wool [36]. Farm Animal contributes significantly to the economy of farmers in the Mediterranean as well as African and Southeast Asian countries [18]. The mass production of small ruminant in the country is constrained by disease, inadequate nutrition, poor genetic resources of the local stock, marketing, social factors, structural constraints and a shortage of high level trained manpower [71]. Mohamed and Abdelsalam, [49] reported that respiratory tract infections were of common occurrence in various species of domestic and farm animals. Pathogenesis is multifactorial, and the diseases appear due to the interaction of infectious micro-organisms (bacteria, mycoplasma, viruses and fungi), host defense, environmental factors and stress [37-39]. Bacterial infection of the respiratory tract may be primary, occurring in healthy individuals or secondary to a large number of conditions which causes immunosuppression [71]. Secondary bacterial infection occurs especially when the resistance of the respiratory mucosa is lowered and bacteria growing in the upper respiratory tract extend downwards [71]. Pneumonia is a major respiratory disease of domestic animals worldwide, especially in countries where livestock management and husbandry are yet to be developed [1, 5]. The disease incidence is usually very high in these areas and this causes serious financial losses to the livestock industry [59]. Among the important infections that are frequently diagnosed in veterinary clinics and abattoirs is pneumonia [7, 24]. Sayed and Zaitoun, [63] pointed out that pneumonia is the most frequently occurring respiratory infections in domestic animals, their aetiologic agents being bacteria, viruses, parasites or concert effect of all of them, often predisposed to by several

factors. Pneumonias can be acute, chronic or progressive [6]. In summary, in Nigeria, some livestock owners dispose of sick, debilitated and infertile animals in an effort to minimize losses, thereby leading to an increase in the risk of slaughtering and consumption of sick animals [4, 52]. There has been little work done on this topic with regards to extensive aerobic bacteria isolation and histopathological examination in Nsukka agroecological zone of Nigeria [17].

Mastitis is one of the major pathogenic microorganism associated with farm animal, it is an inflammation of the udder resulting in a blockage of the milk duct, therefore bringing about physical, chemical and bacteriological changes in milk and pathological changes in the udder [9, 58, 65]. Mastitis is an infection of the tissue of the breast that occurs most frequently during the time of breastfeeding [69]. It is one of the most important and influential single health disorder affecting milk production in dairy farms. It is the disease that affects the dairy industry globally and causes significant economic losses. In the United State of America, the resulting loss due to mastitis in dairy industry is about of $ 2 billion annually [9]. The severe economic losses are due to reduced milk yield; milk quality and early culling of severely affected animals [70]. Also, colossal amounts are expended in antibiotic treatment of mastitis and veterinary services are wasted and economic losses are incurred due to mortality of young animals [8, 9].

Mastitis can be classified as either contagious or environmental mastitis [9, 66, 67]. The former is caused by organisms such as *Staphylococcus aureus* and *Streptococcus agalactiae* that are adapted to survive within the host, particularly in the mammary gland [11, 46, 47]. Environmental mastitis is caused by organisms such as E coli, Streptococcus uberis that do not usually live within the host but enter the teat canal when the cow comes into contact with a contaminated environment. [9, 40] They are common causes of clinical mastitis, with the infection occurring either between milking's such as teat contact with pathogenic material or during the udder preparation before milking [13, 15]. Elsheikh and Hassan [24] stated that Contamination with the teat end of a susceptible quarter with a pathogen is the first stage in the infection processes.

Streptococci were the first bacterial organisms to be incriminated as the cause of contagious mastitis right before the era of antibiotics. *Streptococcus agalactiae* (Lancefield group B), *Streptococcus dysgalactiae* (Lancefield group C) and *Streptococcus uberis* are the main streptococci species involved in mastitis [8, 10, 14]. *Streptococcus agalactiae* is the major cause of chronic mastitis, and is most prevalent in herds [20, 25, 29, 43]. It can survive for long period only within the mammary gland and the effect of infection on udder is devastating. It could result in fibrosis and agalactiae [3, 48].

Environmental Streptococci, *Streptococcus dysgalactiae* and *Streptococcus uberis* cause both subclinical and clinical mastitis [16, 26]. There are forty (40) different species of Staphylococcus, which are divided into coagulase positive and coagulase negative staphylococci (CNS) based on the ability to coagulate plasma [30-34]. Among these, *Staphylococcus aureusis*by far the most pathogenic. It causes chronic and deep infections of the mammary gland that are extremely difficult to cure [50]. Elsewhere, It was reported to be the most frequent and predominant cause of both clinical and subclinical mastitis [41, 51].

Coagulase negative staphylococci (CNS) were traditionally considered minor pathogens ornon- pathogenic as infections with these organisms mostly remaining subclinical. However, they may cause persistent infection that can damage the udder. Some authors have reported a high percentage of clinical cases of mastitis evoked by CNS [11, 46, 47]. Escherichia coli produce endotoxin, which is responsible for many of the observed inflammatory mammary and systemic changes. Cases of coliform mastitis are usually pre-acute, acute or chronic in nature and may cause death [35, 65]. Chronic clinical mastitis is characterized by periodic subacute to acute flare-ups that may persist for months [12]. Certain factors influence the prevalence of mastitis; such as age, parity numbers and stage of lactations where most of the new infections occur during the early parts of the dry period (little milk production) and in the first 2 months of lactation [28, 55-57].

In most countries, surveys of dairy herds indicate that there is relatively high incidence of clinical mastitis and, many farms have high prevalence of subclinical mastitis too [53]. Prevalence with infection of mastitis pathogens is about 50% in cows and quarter infection rate of about 25% [60]. In Nigeria, Rylatt, Wyatt, and Bundesen, [61] reported a prevalence rate of 30% and 3.2% for *Staphylococcus aureus* from settled and traditionally nomadic herds, receptively, in Birnin Kebbi. In another study by Bala, Garba, and Yazah, [4] an incidence rate of 55.4% for *Streptococcus uberis*, 24.6% *Streptococcus agalactiae* and 12.3%*Streptococcus dysgalactiae* was reported in Ibadan [27]. Prevalence rate of 25.7% *Staphylococcus aureus*, 15.4% *Streptococcus agalactiae* and 14.1%. *Escherichia coli* in bovine mastitis had also been reported [42], in Tanzania.

2. Methods

2.1. Study Animals

The Animals used on this research were Ram, Sheep, Goat and Cow.

2.2. Sample Collection

The isolates were collected in a slant bottle (Stock culture) and are labeled accordingly before the identification.

2.3. Sub-Culturing of the Isolate

The pathogens isolated from the animals collected in stock (slant bottles). Each of the isolate was sub-cultured into a prepared nutrient agar plate by streak method of inoculation after which they were incubated for 24hour at 37°C before that identification.

2.4. Identification of the Isolates

The identification process was carried out by inoculating the organism into various selective and differential media

namely, Eosin Metlyene agar (E.M.B), Salmonella shigella (S.S) and Macconkey agar medium this was then followed microscopic examination and Biochemical test Catalase, Oxidases, indole urea e.t.c).

2.4.1. E.M.B

The pathogend subculture into prepared EMB agar plate Using spread method of inoculation. After which the plate was inoculated for 24hours at 370°C macroscopic examination was carried out. *E.coli* appear green metallic sheen while *E. aerogenes* appears pinkish in colour.

2.4.2. S.S

The same procedure was carried out for S.S agar, Microscopic examination of the pathogens shows that salmonella appears colourless in colour with back dot at the center. While Shigella appear only colourless.

2.4.3. Macconkey Agar

After inoculating the pathogen is Macconkey agar medium, *Staph* sppappear pale pink in colour, E. coli red, and Enterococcus appears red in colour.

2.5. Microscopic Examination

Microscopic examination was carried out by gramstain techniques, to know whether the pathogens are gram + Ve or- Ve and to confirmed the pathogens isolated.

2.6. Smear Making

A drop of distilled water was place at the center of the slide, the loopfull of the bacteria isolated was pick and emulsified on the drop of the water to make a thin smear. The smear was heat fixed and allowed for a day.

2.7. Gram Staining and Microscopy

The Gram staining procedure modified by Rueckert and Morgan, [60] was used in this research work. Gram staining clean glass slides were obtained and using the sterile technique a smear of each of the microorganism from 18 – 28 hours culture (while micro-organism were still young) was prepared, and heat-fixed. The smear was gently stained with basic dye crystal violet and left for 1-2 minutes. This was then rinsed rapidly with water, followed by treatment with gram's iodine solution and left for 1 minute which increased interaction between the bacteria cell and the dye so that the dye was more tightly bound or the cell was more strongly stained. The iodine was then poured off, blotted and the slide or smear decolourized by washing with 95% ethanol until no more stain ran from the side. The slide was then washed well with water and stained with safranin for 30 seconds, which was then washed well and dried. Preliminary characterization bygram staining was done (using safranin) on each of the isolated using the method [12] and observed under a light microscope at X 100 and this was observed under oil immersion. The gram staining technique was used to categorize the isolated into gram negative and gram positive [18, 44-45].

2.8. Biochemical Test for Pathogenic Bacteria

Catalase Test

A colony of 24 h old culture was picked using a sterile loop and then emulsified in a few drop of hydrogen peroxide on a clean slide. Presence of effervescence indicated catalase positive reaction whereas negative reaction showed no effervescence [62, 64].

Indole test

The sterile wire loop was used to inoculate organism in a test tube containing 5 ml of peptone water (medium) and incubated for 48 h at 37°C. After incubation, 0.5 ml of Kovac's reagent was added into the tube and allowed to stand for 15 minutes. A rose spank colour indicated positive reaction [62].

Coagulase Test

Two to three drops of plasma were dropped onto the real bacterial suspension. Immediate coarse clumping of the mixture within 5-10 seconds indicate positive coagulase test [62].

Oxidase Test

A piece of filter paper was soaked with oxidase reagent, a colony of test organism is then smeared on filter paper deep purple color indicate that phenylene diamine in the reagent oxidize by the oxidase in the test organism [19, 62].

Urease Test

A well-isolated colony was picked from the surface of the medium and the inoculated as single streak on the slant surface of Christensen's urea agar. The pH shift was detected by the color change of phenol red from light orange to magenta which indicated a positive result. [12, 23].

3. Results

Table 1. *Sources of Isolates and their Code from farm animal.*

S/No	Sources of isolates from farm animal	Isolates codes
1.	Vagina Goat	VRG
2.	Female Goat Anus	FGA
3.	Stool Goat	STG
4.	Male Goat Anus	MGA
5.	Goat Urine	URG
6.	Udder Goat	UDG
7.	Penis Sheep	PEG
8.	Vagina Sheep	VIS
9.	Female Sheep Anus	FSA
10.	Stool Sheep	STS
11.	Male Sheep Anus	MSA
12.	Sheep Urine	URS
13.	Udder Sheep	UDS
14.	Penis Sheep	PES
15.	Vagina cow	VRG
16.	Female cow anus	FCA
17.	Stool cow	STC
18.	Male cow anus	MCA
19.	Cow urine	URC
20.	Udder cow	UDC
21.	Penis cow	PEC

Table 2. *Colony Characteristics and Biochemical Properties of Bacteria Isolated from Farm Animals.*

ISOLATE CODE	MICROSCOPY	MICROSCOPY	MO	CA	IND	GL	LAC	MA	COA	AT	OXI	UR	MR	VR	BACTERIA ISOLATED
VRG	Small circular colonies	Gram-ve rod	+	+	+	+	+	+	-	-	-	-	+	-	*Escherichia coli*
FGA*	Flat circular milky colonies	Gram +ve cocci in Clusters	-	+	-	+	-	-	+	-	-	-	+	-	*Staphylococcus aureus*
URG*	Small circular colonies	Gram-ve Rod in chain	-	+	+	-	-	-	+	-	-	-	+	-	*Shigellasp*
STG*	Small circular colonies	Gram -ve	+	+	-	+	-	+	-	+	-	-	+	-	*Salmonella sp*
UDG*	Flat circular milky colonies	Gram + ve cocci in chain	-	-	-	+	-	-	-	-	-	-	-	+	*Streptococcus agalactiae*
PEG	Small circular colonies	Gram-ve rod	+	+	+	+	+	+	-	-	-	-	+	-	*Escherichia coli*
MGA	Small circular colonies	Gram-ve rod	+	+	+	+	+	+	-	-	-	-	+	-	*Escherichia coli*
VIS*	Flat circular milky colonies	Gram + ve cocci in chain	-	-	-	+	-	-	-	-	-	-	-	+	*Streptococcus agalactiae*
STS*	Small circular colonies	Gram-ve Rod in chain	-	+	+	-	-	-	+	-	-	-	+	-	*Shigellasp*
MSA*	Small circular colonies	Gram-ve Rod in chain	-	+	+	-	-	-	+	-	-	-	+	-	*Shigellasp*
URS*	Small circular colonies	Gram -ve	+	+	-	+	-	+	-	+	-	-	+	-	*Salmonella sp*
UDS	Flat circular milky colonies	Gram +ve cocci in Clusters	-	+	-	+	-	-	+	-	-	-	+	-	*Staphylococcus aureus*
PES*	Small circular colonies	Gram-ve rod	+	+	+	+	+	+	-	-	-	-	+	-	*Escherichia coli*
FSA	Small circular colonies	Gram-ve rod	+	+	+	+	+	+	-	-	-	-	+	-	*Escherichia coli*
VRC*	Small circular colonies	Gram-ve rod	+	+	+	+	+	+	-	-	-	-	+	-	*Escherichia coli*
FCA*	Small circular colonies	Gram –ve	+	+	-	+	-	+	-	+	-	-	+	-	*Salmonella sp*
MCA	Flat circular milky colonies	Gram+ve cocci in Clusters	-	+	-	+	-	-	+	-	-	-	+	-	*Staphylococcus aureus*
STC*	Flat circular milky colonies	Gram +ve cocci in Clusters	-	+	-	+	-	-	+	-	-	-	+	-	*Staphylococcus aureus*
URC*	Small circular colonies	Gram-ve Rod in chain	-	+	+	-	-	-	+	-	-	-	+	-	*Shigellasp*
UDC	Small circular colonies	Gram-ve rod	+	+	+	+	+	+	-	-	-	-	+	-	*Escherichia coli*
PEC*	Small circular colonies	Gram-ve Rod in chain	-	+	+	-	-	-	+	-	-	-	+	-	*Shigellasp*

Keys: MO=MotilityCO=Coagulase IND=Indole GL=GlucoseLAC=Lactose MA=Mannitol CAT=Catalase OXI=Oxidase test, UR=UreaMR=Methyl red, VP=VogesProskauer+ or **(**+ve**)**=Positive- or (-ve)=Negative.

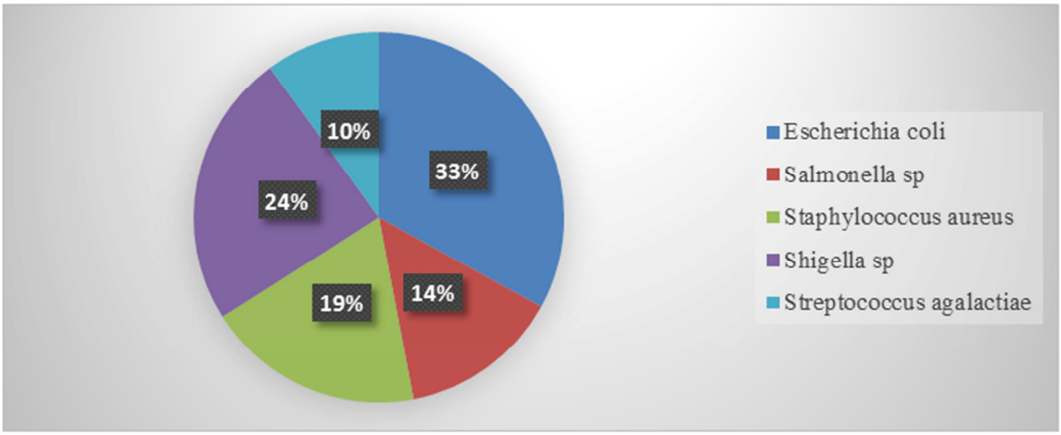

Figure 1. *Percentage of occurrence of pathogens from different farm animal.*

4. Discussion

The sources of the sample are vagina, anus, stool, urine, udder and penis sheep. Fifteen (15) of the samples namely (Vagina swab, anus swab, and penis swab) were collected with the aid of sterile swab sticks while the urine and stool samples were collected in sterile containers then all the samples were subjected to isolation and identification of the microorganisms associated with the samples.

In Table 2, the result of the Biochemical tests performed on the samples 14 out of the 21isolates identify as pathogen. The result revealed the presence of Gram positive bacteria such as *Streptococcus agalactiae,* and *Staphylococcus aureus* as well as Gram negative bacteria such as *Escherichia coli* and *Shigellasp*and *Salmonella sp*in the samples which is in agreement with [45]. They were identified based on their reaction to biochemical tests. This result is in line with the findings of Capasso, [15]. In their research, they stated that microorganisms can be identified based on their reaction to biochemical test and also went further to describe the reactions of these organisms. They stated that any organism that is motile, and react to catalase, indole, and methyl red positively and react to coagulase, oxidase and urease negatively and has the ability to utilize triple sugar (Maltose, glucose, and lactose) is *E. coli*, any organism that is non-motile and react to catalase, indole, coagulase and methyl red positively but react negatively to oxidase and urease and could not utilize the triple sugar is *Shigella dysenterea*, any organism that is motile and react positively to catalase and methyl red and react negatively to coagulase and indole and could utilize triple sugar with the exception of lactose is a *Salmonella* sp, any organism that is non-motile and react positively to catalase, methyl red and coagulase but react negatively to oxidase, indole and urease and could not utilize lactose and maltose is *Staphylococcus aureus* and any organism that reacts to catalase, indole, urease, coagulase, methyl red, and oxidase negatively and have no ability to utilize lactose and maltose is *Streptococcus agalactiae.* The reactions were observed during the biochemical tests.

Figure 1 shows the percentages of occurrence of pathogens in the farm animals *Escherichia coli* has the highest percentage of occurrence (33%) followed by *Shigellasp* (24%) while *Streptococcus agalactiae* has the lowest occurrence of (10%).

Finally, the bacteria isolated from the VIS sample after identification was *Streptococcus agalactia,* from STS and MSA was *Shigellasp,* from URS was *Salmonella sp*, from UDS was *Staphylococcus aureus*, and from PES and FSA were*Escherichia coli*. The present of these pathogenic bacteria in the farm animals implies that the animals used in this research are not healthy and if the meat is consumed by human can lead to food poisoning.

5. Conclusion

It can be concluded that pathogenic bacteria capable of causing food poisoning were isolated from live animals using microbial analysis. Also such bacteria can be identified using appropriate biochemical tests thereby providing information on their microbial quality. The presence of bacterial load can lower the nutrition value of these foods due to food poisoning and some intestinal diseases. Routine microbial analysis of animals most especially sheep in Rugga settlement of Birnin Kebbi be carried out to prevent outbreak of food poisoning and animal diseases. Finally, proper steps should be taken to ensure that the occurrence of such organisms in these animals is kept within limits so as to save the lives of these animals.

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