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Bacteria Isolated From Respiratory Tract and Abscesses in Livestock

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Abstract. This research aims to identify the types of bacteria that have been isolated from various body fluids, including the abscess secretions and nasal secretions of sheep and cows. Cotton swabs were used togather isolates from animals suffering from respiratory ailments and abscesses. The specimens were subsequently transferred to the microbiological laboratory. They were cultured on blood agar media and incubated for a duration of 24 to 48 hours at a temperature of 37°C. Following the purification of the samples, traditional diagnostic methods, including biochemical tests, were conducted. Polymerase chain reaction, Its foundation is the utilization of the 16SrRNA gene which depends genetic material to diagnose bacteria .The 16SrRNA gene, found in all bacterial species, contains nine heterogeneous regions that facilitate the identification of various types of bacteria.. The sequence of the samples is then compared with the sequence found in GenBank, each bacteria has an international number by which it can be identified. The results of isolates obtained from the nasal secretions is Acinetobacter baumannii and from abscess secretions is Staphylococcus agnetis The study revealed that rare specimens were obtained and registered in the gene bank bearing the following numbers , PP809047.1 , PP809049.1. The results of the study also confirmed that some isolates, especially bacteria, are pathogenic and pose a danger to animals, but when transmitted to humans, they are deadly due to their opportunistic nature.

Highlights:

1. Bacteria isolated from sheep and cow secretions: Acinetobacter baumannii, Staphylococcus agnetis.
2. Diagnostic methods: blood agar, biochemical tests, PCR using 16SrRNA gene.
3. Rare specimens registered in GenBank, potentially pathogenic to animals and humans.

Keywords: Respiratory tract infection , Body fluids ,Livestock.

Introduction

Domestic animal production, particularly that of sheep, is already vital to the world's agricultural economy as a whole, providing essential products such as meat, milk, skins, as well as wool. Livestock, such as cattle, are essential to the economy of millions of people throughout the world because they allow farmers to make so many different products and generate so much money. Due to the transition from small family

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enterprises to major corporations, there has been an upsurge in the demand need animal products. Many people are worried about the health and well-being of the animals raised in these farms and the risks they face from diseases like bacterial infections, which can reduce their productivity and the quality of their products [1]. Animals view bacteria as an integral part of their microbial environment due to their presence in numerous physiological systems and their diverse, sometimes beneficial functions. Beneficial bacteria aid digestion and strengthen the immune system when conditions are optimal. However, once there is equilibrium or pollution, harmful microbes can cause serious infections and a host of other maladies that negatively impact the animal's health. As a result, assessing the presence of pathogenic microorganisms in sheep's internal fluids is essential for administering appropriate care and maintaining the herd's health [2]. It is crucial to isolate and categorise bacteria from sheep body fluids in order to detect harmful bacterial species and learn how they develop treatment resistance. The idea is that this procedure can help us better understand the dynamics of diseases and develop more effective treatments. In order to detect and diagnose bacterial secretions, it is necessary to collect samples from various body fluids, including blood, urine, dairy products, wound fluids, even respiratory fluids. The bacteria can be identified at a preliminary stage based on their growth traits and morphology after being cultured as well as separated on particular nutritional media [3].

A variety of laboratory procedures are required for the isolation and identification of bacteria from body fluids, including: bacterial culture, in order to grow and identify bacteria, samples are cultured on particular nutrient media. The shape and growth features of bacteria can be used as first identification criteria in this method [4].

Biochemical tests to identify microbes by analysing their various chemical responses. Molecular diagnostic methods: for example, the polymerase chain reaction (PCR), that utilises DNA analysis to reliably and precisely identify bacteria. In addition, the precise details regarding bacterial genetic makeup that genomic sequencing offers aids in deducing the origins of treatment resistance [5].

After the bacteria have been discovered, the most effective course of treatment is to determine their susceptibility to a specific types of antibiotics [6].

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Methods

Sampling

From October 2023 to February 2024, a range of physiological fluids were collected from animals, particularly sheep, at several sites. These included:

1. The Al-Qurna District 1 Veterinary Medicine Centre
2. The veterinary clinics are located in the Al-Qurna district

For the purpose of bacterial isolation, cotton swabs were taken from the mouths and noses of sheep that were sick with respiratory diseases. Samples were transferred to 5the laboratory of microbiology at the University of Basrah

Bacterial Isolation

After transferring the samples to the laboratory of microbiology, they were cultured on blood agar and MacConkey agar,Then incubate for 24 to 48 hours at 37°C. Bacterial colonies then purified by spreading method on blood agar medium.

Traditional Methods

Gram stain

Pure colonies were made and the gram stain procedure was conducted in order to determine if the material tested positive or negative.

Biochemical test

These tests include the following biochemical assays, which depend on the ability of bacterial isolates to produce enzymes such as catalase, Oxidase, Urease, in addition to other biochemical.

PCR Technique

Extraction of DNA

Using extraction kits The Presto TM Mini gDNA Bacteria Kit, supplied by Geneaid, was used to separate DNA from pure bacterial isolates. According to the manufacturer's directions.

Detection of 16srRNA gene using PCR

Was discovered using the PCR method, and a primer was required according to the researcher's methodology [7]. The primer sequence is shown in the Tables (1,2, and 3).

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TABLE 1. Universal 16SrRNA primers.

Primer	Sequence	Product size
Forward	5′-AGAGTTTGATCCTGGCTCAG-3′	1500
Reverse	5′-GGTTACCTTGTTACGACTT-3′	Base pair

TABLE 2. Reagents of PCR amplification (25µl) for 16srRNA gene.

Components	Concentration
PCR Master mix	5µl
Forward primer	1µl
Reveres primer	1µl
DNA	1.5µl
Nuclease – free water	16.5µl
Final volume	25µl

TABLE 3. PCR condition for amplifying 16SrRNA.

Phase	Tm (c)	Time	No. of cycle
Initial DNA Denaturation	94c	5 min	1 cycle
DNA Denaturation	94c	40sec	33cycle
Annealing	50c	40sec	
Extention	72c	1.30min	
Final Extention	72c	7min	1 cycle

Agarose gel electrophoresis

After 5µl of the material has been extracted and placed onto an agarose gel that has been previously prepared using the researcher's methods, the PCR-amplification of DNA is completed. This makes it possible to calculate the amplified gene's size [8].

Result and Discussion

After the sample was fully purified, a gram dye was made on the isolates, and the result was positive to Staphylococcus agnetis, but the result was negative to Acinetobacter baumannii as illustrated in Table (4).

TABLE 4. The results of biochemical reactions of samples.

Biochemical tests	Catalase	Oxidase	Urease	Citrate	Methyl red	Indole
Acinetobacter baumannii.	+	-	+	+	+	-

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Staphylococcus agnetis	+	+	+	-	-	+

Biochemical examination of the bacteria produced the following results: all samples tested the catalase test resulted in bubbles forming when the reagent was added, showing that the bacteria were positive for the test. The medium changed from yellow to pink in the urea test, suggesting that the bacteria could produce the urease. This test likewise yielded a favorable result. The medium changes from green to blue, signifying that the Acinetobacter baumanni bacteria are positive for the test, proving that the bacteria were tested for utilization. The oxidase test produced a negative result since there was no color change visible after adding the reagent, while the red Methyl red test produced a positive result because a red ring formed at the top of the surface, demonstrating the ability of the bacteria to produce the enzyme. The indole test yielded a negative result as well because a yellow ring was visible at the surface. A sign that the bacteria are harmful. After introducing the reagent to the staphylococcus agnetis bacteria, a purple tint was seen, demonstrating the bacterial ability to produce the oxidase enzyme. This indicated that the bacteria tested positive for the oxidase test. It was found that the surface remained green in the citrate test, indicating that the bacteria were unable to utilization of citrate. However, it was positive for indole, as a red ring formed at the top of the surface, indicating the ability of the bacteria to produce the indole enzyme. Additionally, the results of the red proverb test were negative, showing that the bacteria were unable to generate the enzyme as seen by the yellow ring that formed at the surface [9].

As can be seen in the Figure (Polymerase chain reaction product of the 16SrRNA gene electrophoresis for bacterial isolates)

, the 16SrRNA gene's polymerase chain reaction revealed that it contains 1500 base pairs when compared to the size guide DNA ladder. Consequently, three bacterial isolates that were supposedly diagnosed underwent partial diagnosis to confirm the kind of sample. The study's findings were in agreement with [10] which used this gene to diagnose bacteria since it contains known molecular packages. Furthermore, [11] discovered bacteria that utilized the same gene.

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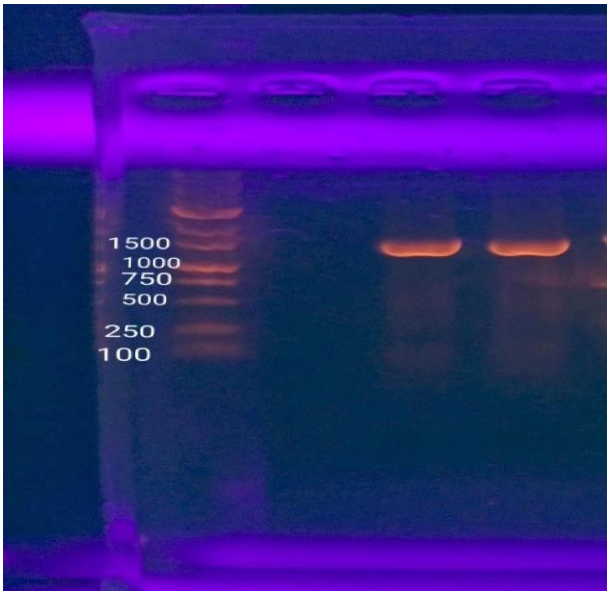

FIGURE 1. Polymerase chain reaction product of the 16SrRNA gene electrophoresis for bacterial isolates.

The fundamental structural component of the 30s subunit in early ribosomes is the s in 16SrRNA. The acidic sequence known as rRNA encodes rRNA, which is present in all types of bacteria. All bacteria share the conserved and heterogeneous regions that comprise the gene's internal structure, but the latter vary to varying degrees [13]

Since all kinds of bacteria have the 16SrRNA gene, the PCR amplification method was utilized to diagnose the bacterium. Species and genera can be identified thanks to the gene. It is made up of multiple different nucleotides (Jenkins et al.).

The sequences were examined using the Sanker method after a BLAST search on the National Center for Biotechnology Information website produced a high match with reference species and genera in GenBank. Due to their rarity, the samples were recorded under the following GenBank numbers. PP809047.1, PP809049.1

The drug susceptibility test used antibiotic, and the results indicated that the isolates exhibited very high resistance to a variety of antibiotics, including ampicillin, metronidazole, and ampicillin

Conclusion

The bacteria originating from sheep pose a significant threat; however, when transmitted to humans, they can act as opportunistic pathogens with the potential to be

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lethal to the host. Their ability to resist a broad spectrum of medications is exacerbated by the presence of various virulence factors

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