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Detection *Scl1* gene of *Streptococcus pyogenes* isolated from respiratory infection of local goats breed

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Abstract

This study aimed to detect the molecular characterization of *Streptococcus pyogenes* (*S. pyogenes*) that isolated from respiratory diseases in local goats breed in Najaf province. The research was done during one year period from April (2012 and 2013). Eighty-seven nasal swabs were taken from clinically infected goats, which showed typical respiratory signs such as nasal discharge, cough, fever, dyspnea and ocular mucopurulent discharge. Isolation and identification of *S. pyogenes* were determined by the classical microbiological techniques. Seventy-six (87.3%) bacterial isolates were grown on blood agar with Bacitracin disk. Out of 76 bacterial isolates, 48 (63%) were shown Beta-hemolysis and sensitivity to bacitracin disk while, 28 (36.8%) were resistance to bacitracin and revealed different hemolytic types. The results of molecular investigations confirmed the presence of streptococcal collagen-like protein (Scl1) gene in 7 (14.5%) out of 48 isolates by conventional PCR technique.

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Introduction

Small ruminants respiratory diseases are multifactorial. These respiratory diseases are caused by numerous etiological agents (Lacasta *et al.*, 2008). These bacterial diseases have specific important attributable to several clinical signs, a severity of diseases, and some strains resistant to many of antimicrobial agents (Woldemeskel *et al.*, 2002). The respiratory diseases formed 5.6 % of all these diseases in small ruminants (Hindson and Winter, 2002). Pneumonia is caused by several infectious and

non- infectious agents. The infectious agents are included bacteria, viruses and fungi (Garoia *et al.,* 1998). Pneumonia is one of the most important diseases that cause death in goats (Ameh *et al.,* 2000). Respiratory diseases have great economic concern for goat producers around the world. Pneumonia causes significant economic losses such as the death of animals, weight loss, poor quality meat and recognition of the infected lung during the inspection (Al–Rawashdeh & Al–Qudah, 2000). *Staphylococci* and *Streptococci* are group of bacteria that cause a wide range of diseases such as pneumonia, septicemia, endocarditis and other diseases. It causes of nosocomial and community-acquired infections (Craciunas *et al.,* 2010). *S. pyogenes* causes various disease, such as pharyngitis, cellulitis, and bacteremia (Stevens, 1998). The streptococcal collagen-like protein (Scl) gene in the *S. pyogenes* cell surface protein family, has been identified recently (Rasmussen *et al.,* 2000; Whatmore, 2001).

There is a shortage in the molecular studies regarding the molecular characterization of the causative agents of the respiratory diseases of goats in Iraq. This study intends to detect the molecular features of *S. pyogenes* that isolated from respiratory diseases in local goats breed in Najaf province/ Iraq.

Materials and methods

Sample collection

The research was carried out between April 2012 and 2013. Totally, 87 nasal passages swabs were collected from the infected goats in different villages and townships of Al-Najaf Governorate (AL-Manthera, Al-barkat, Al-hira, Al akarate). The swabs were put into the test tubes that containing transport medium (3 ml of Tryptone soy broth) and transfer directly into a cooler box to the laboratory.

Bacteriological processing

Swabs were processed according to the routine bacteriological methods. All samples were cultured onto 5 % sheep blood agar with a low concentration of bacitracin disc (0.025 IU). The plates were incubated at 37 C in a candle jar incubated for 18-24 hr. Later on, the bacterial colonies were examined for beta-hemolysis, gram stain and its resistance or sensitivity for bacitracin disc (Vandepitte, 2003).

DNA Isolation and Purification

Bacterial genomic DNA was isolated by using the Wizard Genomic DNA Purification Kit (Promega, USA). Nucleotide sequences of primer and the expected sizes of PCR product was 181 bp. Scl1-1 gaacctcgtgtgccagaaaa, Scl1-2 cgaaggcttgcatgtaacct were designed for this study. The Primer was provided from (Bioneer, Korea). The Scl1 gene (streptococcal collagen-like) were amplified using 3 µl of *streptococcal* DNA. The 25 µl -PCR mixture contained 0.5mM of the primer, 200 mM of each deoxyribonucleoside triphosphate (Eurobio, France), 3 mM MgCl2, and 1 U of Taq

DNA polymerase in 1X buffer according to the manufacturer's instructions (Promega).

DNA Amplification

The DNA was amplified according to routine PCR protocol using conventional PCR thermocycler system. The amplification steps were : Step 1: 95ºC, 2 min. Step 2: 95ºC, 30 sec. Step 3: 54.2ºC, 30 sec. Step 4: 72ºC, 20.0 sec, Step 5: Repeat steps 2-4 for 29 more times, Step 6: 72ºC, 5 min, Step 7: 4ºC. Finally, the prepared PCR mixture were analyzed by electrophoresis through a 1% agarose gel in 1 X TBE buffer with ethidium bromide and visualized using UV-Gel Doc system (Bio- Rad).

Statistical analysis

Chi-square (x^2) test was used to analysis the level of significance at $p<0.05$ (Niazi, 2000).

Results

The clinical examination of the infected goats, revealed nasal discharge, cough, fever, dyspnea and ocular mucopurulent discharge. The distribution of bacterial isolates, according to regions, were 90%, 88%, 86% and 85 % at AL-Manthera, Al-barkat, Al-hira and Al-akarate respectively (Table 1). In addition, no significant differences were seen at ($p< 0.05$).

Table 1. Showing the geographical distribution

Region	No.nasal swab	Positive growth	%
AL-Manthera	20	18	90
Al barkat	17	15	88
Al-hira	23	20	86
Al akarate	27	23	85
Total	87	76	

*No significant differences at ($p< 0.05$)

Identification and biochemical characterization

On the blood agar, 76 nasal samples were grown, with the percentage of (87.3%). The percentages of bacterial isolates that revealed Gram-positive, beta hemolysis and sensitive to bacitracin disk, were 48 (63%). However, 28 (36%) isolates were revealed variable hemolytic characteristics and most of them were resistance to bacitracin and Gram negative (Table 2).

Table 2. Shows the isolation and characterization of *S. pyogenes* using classical methods.

Positive growth		Hemolytic characteristics	Bacitracin test	Gram stain	%
76	48	Beta	Sensitive	Variable + (-)	63
	28	Others	Resistance	_	36

Molecular characterization

Bacterial genomic DNA were extracted from 48 isolates that cultred for overnight in the broth. The amplification of 181 bp product size of (Scl1) gene of the 48 isolates, were confirmed in 7 isolates as *streptococcus pyogenes* in conventional PCR technique. These bacterial isolates (7 out of 48) were carrying streptococcal collagen like protein (*Scl gene*) of *S. pyogenes* (Table. 3 and Figure. 1).

Table 3. Shows the detection *S. pyogenes* by molecular PCR technique and conventional method.

No. positive bacteria	Bacterial conventional methods	PCR technique Scl1
76	48 (63.1%)	7 (14.5%)

Discussion

Respiratory diseases of sheep and goat may be affecting individuals or groups and may cause a high rate of mortality in case of poor live weight gain (Kumar *et al.,* 2000). In the present study, the incidence of pneumonic *S. pyogene* revealed high percentages (14.5%). The result of this study is higher than results reported previously (Obasi *et al.,* 2001). Obasi *et al.,* (2001), which recorded the occurrence of ovine and caprine bacterial pneumonia in Nigeria during 10 years. They found that *S. pyogenes* occurred at 6.9% rate, moreover the prevalence of ovine and caprine pneumonia caused by *S. pyogenes* was (8.3%) and (2.5%) in Zaria and Nigeria respectively (Raji *et al.,* 2000). Among Bighorn Sheep, low isolation percentage of *Streptococcus spp*. in Western United States, was also reported by Thomas *et al.,* (2012).

The significant variation between the results of this study and previous studies are attributable to sensitivity and specificity of the tools, which were used in the isolation and identification of the causative agent. Moreover, PCR considered as a gold standard technique in compare to classical bacteriological methods that used in the previous studies.

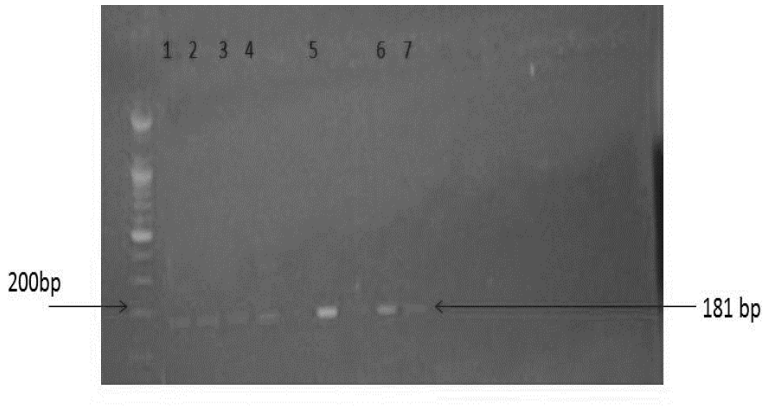

Figure .1: Shows the gel electrophoresis of DNA fragments 181 bp amplified fragment of *Scl1* gene among the examined *S. pyogenes* isolates and the 200bp DNA ladder.

There is a lack of available research and data about the molecular-based diagnosis technique of *S. pyogenes* in animals, except some studies (Tushar *et al.,* 2012). Tushar *et al* (2012), detected the molecular features of *streptococcus spp*. at (14.3%) from healthy as well as sick goats in India. In this study, the result of a molecular analysis, was less than the percentage of previous studies (Tijjani *et al.,* 2012). Tijjani *et al.,* (2012), found that the incidence of *S. pyogene* was (19.6%) in pneumonic goat in Nigeria. However, Legesse *et al.,* (2010), found a high proportion (55 %) of mixed infection of *Streptococcus* and *Staphylococcus* that isolated from lungs of sheep in Ethiopia. Increase the incidence of pneumonia may be due to the environmental factors that cause an increase in the virulence of the pathogen and make the animal more susceptible (Kuehn *et al.,* 2005; Jelsbak *et al.,* 2012; Mart´ınez and Baquero, 2002). In conclusion, this study, approved that the molecular diagnostics technique was used as a diagnostic method for detection *S. Pyogenes* that isolated from goat respiratory disease. The author recommends using the molecular diagnostic technique to detect *S. pyogenes* genes and even in choose the correct antimicrobial therapy.

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