

*Full Length Research Paper*

# Isolation and identification of *Archanobacterium pyogenes* (*Actinomyces pyogenes*) from Arabian gazelles

Mohammed S. Al-Saggaf\*, Rashead M. Al-ssum and Omar M. H. Shair

King Khalid Wildlife Research Center, Riyadh, Saudi Arabia.

Accepted 21 September, 2011

**Archanobacterium pyogene** (*Actinomyces pyogene*) is an opportunistic pathogen of economically important livestock such as dairy, beef cattle and gazelles. It is also a common inhabitant of the mucous membranes of these animals. This study was aimed to investigate the epidemiology of *A. pyogenes* in the infected Arabian gazelles kept at King Khalid Wildlife Research Center at Thumamah, 70 km north of Riyadh, Saudi Arabia and the relationship between symptoms and infection by *A. pyogenes*. Samples were collected from pleural fluid of 70 infected gazelles and from upper respiratory tract of 30 healthy animals. Enrichment and isolation from the collected samples resulted in 48 bacterial strains, 8 strains from upper respiratory tract of healthy animals and 40 from infected gazelles. Biochemical tests of the 48 isolated bacterial strains revealed that all the strains were beta-hemolytic on blood agar, hydrolyzed gelatin and starch were unable to hydrolyze esculin or reduce nitrate and did not produce urease which was typical characteristics of *A. pyogene*. Identification of the isolated strains was further confirmed by molecular method using analysis of 16S rDNA.

**Key words:** Opportunistic, pathogen, wildlife, Arabian gazelles, *Actinomyces pyogenes*.

## INTRODUCTION

*Archanobacterium pyogene* (*Actinomyces pyogene*) is an anaerobic Gram-positive bacilli and it is a part of the normal flora in many domestic animals (Carlos Emilos et al., 2009). *A. pyogenes* is a rare cause of pyogenic infections in humans and in most of cases; reports are questionable since there was failure to definitively identify the pathogen (Hermida Ameijeras A et al., 2004). *Gazelles* belong to Kingdom Animalia, Phylum, Chordata, Class, Mammalia, Order, Artiodactyla, Family, Bovidae, and Genus Gazelle. Three species of *gazelles* are considered native to the Kingdom of Saudi Arabia including sand gazelles *Gazella subgutturosa marica*, Arabian mountain gazelles, *Gazella gazelle* and the Saudi gazelle *Gazella dorcas* (saudiya). However, the distribution of gazelles in Saudi Arabia has declined dramatically during recent decades due to excessive hunting and habitat degradation. Today, Gazelles

survive only in small numbers in a few isolated parts of their former range. Therefore, King Khalid Wildlife Research Center (KKWRC) was established in 1986 by the National Commission for Wildlife Conservation and Development (NCWCD) to breed native Arabian *Gazelles* species as part of the conservation program for the Kingdom of Saudi Arabia. The center was developed at Thumamah, 70 km north of Riyadh, on the farm of the late King Khalid, whose animal collection formed the basis of the breeding stock. One year later, the management of the animal collection was given to the Zoological Society of London on behalf of the (NCWCD). Different species of *gazelles* kept at the center are for breeding, research and reintroduction purposes.

Two main species of *gazelles* are kept at the KKWRC including, *G. gazella*, locally known as Idmi and *G. subgutturosa marica*, locally known as Reem. Previous study identified, determined the susceptibility and detected gene cassettes of *A. pyogenes* isolates from cows with endometritis (Liu, et al., 2009). It is a commensal and an opportunistic pathogen of

\*Corresponding author. E-mail: [dr.m.alsaggaf@hotmail.com](mailto:dr.m.alsaggaf@hotmail.com).  
Tel: +966 1 404 4412. Fax: +966 1 401 1527.

economically important livestock, causing diseases as diverse as mastitis, liver abscessation and pneumoni (Ceribasi, et al., 2009). Clinical and pathological features of three clinical cases of testicular abnormalities associated with *A. pyogenes*, cellulites and testicular degeneration, confirm that *A. pyogenes* can be a testicular pathogen under field conditions (Gouletsou et al., 2006). Intrauterine inoculation of nulliparous ewes with *A. pyogenes* and *Escherichia coli* produced an antibody response and reduced the severity of subsequent infections (Meghan et al., 2005). *A. pyogene* possesses a number of virulence factors that contribute to its pathogenic potential and expresses a cholesterol-dependent cytolysin, pyolysin, which is a haemolysin and is cytolytic for immune cells, including macrophages (Jost and Billington, 2005). Therefore, more insight on pathogenic sequence of *A. pyogenes* is curial for understanding its physio-pathogenicity. The sequence data from autosequencers, mining Web-databases, performing automatic and manual sequence alignment, analyzing sequence facilitates the promotion and better understanding of the underlying assumptions (Tamura et al., 2007). Accurate identification of bacterial isolates is an essential task in clinical microbiology. Fatal *A. pyogenes* endocarditis case in human, mostly related to living in rural areas and to contacts with animals has been described by DNA sequence (Plamondon et al., 2007). However, phenotypic methods are time-consuming and either fails to identify some bacteria such as Gram-positive rods entirely or at least fail to do so in some clinical situations (Bosshard et al., 2003). The traditional identification of bacteria on the basis of phenotypic characteristics is generally not as accurate as identification based on genotypic methods (Clarridge et al., 2004).

Recombinant DNA (rDNA) sequencing is an effective means for the identification of aerobic Gram-positive rods which are difficult to identify by conventional techniques. 16S rDNA sequencing is a recent method of identification which offers a useful alternative (Mignard and Flandrois, 2006). Partial 16S rDNA was amplified (by using primers p27f and BAC1401r and sequenced by using primers 1100r, 765fs and 10f. Sequences were compared with those available in GenBank using gapped BLASTN 2.0.5 software (Carlos et al., 2009). The objectives of this study was to investigate the epidemiology of *A. pyogenes* in the infected Arabian gazelles under captive at the KKWRC, investigation of the relationship between symptoms and infection by *A. pyogenes*, and determination of the virulence factors encoding genes of the isolated *A. pyogenes*. It is usually recommended to compare this molecular identification tool to classical phenotypic methods. The great potential of the method has been reported for Gram-positive rods and coryneiform bacterial identification (Bosshard et al., 2003) Therefore, 16S rDNA gene sequencing was used in this study to confirm the identification of the bacterial strains isolated from different infected and healthy gazelles.

## MATERIALS AND METHODS

Materials used include captive healthy gazelles at King Khalid Wildlife Research Center, breeding pens, infected gazelles, pleural fluid gazelles, syringes and transport media, as well as specimens of upper respiratory tract of 30 healthy gazelles, 15 Idmi and 15 Reem gazelles.

### Reagents and experimental instruments

sterile saline, 5% sheep blood agar medium and Brain heart infusion (BHI) agar media, sterile loop, 5% CO<sub>2</sub>, Trypticase Soy Broth medium (TSB), 5% fetal bovine serum, 10% (v/v) sterile glycerol, API CORYNE kit (Biomérieux, France), soft agar media, conventional Gram stain, distilled water, buffered glutaraldehyde solution (2.5% glutaraldehyde in 0.05 M sodium cacodylate buffer pH 7.4), phosphate buffer, osmium tetroxide solution (1.0% osmium tetroxide in 0.05 M sodium cacodylate buffer, pH 7.4) and graded ethanol series (25, 50, 75, and two 100% each for 10 min; 5 × 10 = 50 min). In addition, swabs coated with gold, methanolic uranyl acetate solution, Reynolds lead citrate, mineral oil, GP medium, Blood & Tissue Kits (Qiagen, NY, USA), 0.2 μM nucleopore filters.

For DNA sequencing, the following were used; proteinase K, ethanol 96-100%, 20 mM Tris·Cl (pH 8), 0.2 mM sodium EDTA, 1.2% Triton® X-100, lysozyme, agarose, loading dye solution (Qiagen, NY, USA), One kb DNA ladder (Roche, Mannheim, Germany), ethidium bromide solution (1 μg/ml), 50x TAE (Tris-Acetate/EDTA) buffer stock solution, universal eubacterial forward primer 16F27 (5'-AGA GTT TGA TCC TGG CTC AG-3') and reverse primer 16R1525 (5'-AAG GAG GTG ATC CAG CCG CA-3'), MWG Biotech AG (Ebersberg, Germany), deoxynucleoside triphosphates, MgCl<sub>2</sub>, Taq polymerase and 10x PCR buffer. More also, instruments used include; dissecting microscope, scanning electron microscope (SEM), Transmission Electron Microscope (TEM), pH indicator, DNeasy Mini spin column, micro centrifuge tube, vortexes, pipette

### Isolation of *A. pyogene*

Specimens were collected from pleural fluid of 70 infected gazelles. Specimens were also collected from upper respiratory tract of 30 healthy gazelles (15 Idmi and 15 Reem gazelles). Samples were kept in cold and transferred directly to the lab within 1 h for further analysis. Enrichment and isolation of *A. pyogenes* was carried out according to the methods reported by (Ertas et al., 2003). Pleural fluid samples from the abscessed lungs were serially diluted in sterile saline solution up to 10<sup>-4</sup>. Aliquots (200 μL) and swabs (collected from infected and healthy gazelles) were inoculated (in triplicates) onto 5% sheep blood agar medium and Brain heart infusion (BHI) agar media supplemented with 5% sheep blood using sterile loop and incubated aerobically and in 5% CO<sub>2</sub> at 37°C for 72 to 96 h. The obtained single colonies were sub-cultured several times in fresh agar plates until pure homogeneous single colonies were obtained. The cells of the isolated bacteria were then grown in the trypticase Soy Broth medium (TSB) supplemented with 5% fetal bovine serum and incubated at 37°C for 18 h.

Identification of the isolated bacteria was carried out using conventional methods, including morphological, biochemical tests, API CORYNE kit (Biomérieux, France) and was finally confirmed by molecular methods using analysis of 16S rDNA sequence as previously reported. Colony morphology of 24- to 48-h old cultures was examined under a dissecting microscope. Hemolytic activity was recorded on 5% sheep blood agar media after incubation at 37°C for 72 h. Cell motility was examined using soft agar media according to the method described by (Harmon et al., 2005). 3 μL of

overnight culture of the isolates were inoculated carefully to avoid any disturbance of the agar surface, on the center of soft agar medium (0.4%). The plates were then incubated at 37°C and monitored up to 72 h. Motile bacteria migrated from the plate center and diffuse into the medium causing turbidity, whilst non-motile bacteria show growth confined to the center.

### Identification of cell morphology

Cell morphology and cell wall type were examined using conventional Gram stain and the scanning electron microscope. The cell morphology of the bacterial isolates was examined under SEM in the Electron Microscopy Unit available in the National Research Center, Faculty of Science, King Saud University as follows (Allan-Wojtas et al., 2010): The API Coryne kits contained API Coryne strips, GP medium, McFarland standard, nitrate A, nitrate B, Zym A, Zym B, PYZ, H<sub>2</sub>O<sub>2</sub>, mineral oil and sterile saline 3 ml. The procedures of strip inoculation and results interpretation were carried out according to the manufacturer's instructions and the results were interpreted according to the API guideline.

### Gene sequencing analysis

The isolated bacterial strains were further identified using 16S-rDNA sequence analysis. First, the total bacterial DNA was extracted, then the 16S-rDNA gene was PCR amplified, sequenced and the obtained sequences were compared with the sequences of known bacteria in the GenBank database.

### DNA extraction and purification

All the isolates were inoculated into TSB medium supplemented with 5% fetal bovine serum and incubated overnight at 37°C. 3 ml of growth cultures were centrifuged and the pellets were washed twice with sterile saline solution. The total bacterial total DNA was extracted using DNeasy Blood & Tissue Kits (Qiagen, NY, USA) with a resultant yield of 10 µg DNA. The total extracted DNA was subjected to electrophoresis with 1 × TAE in agarose gel by loading (12 µL) mixed with 3 µL of loading dye solution (Qiagen, NY, USA) into the gel wells. One kb DNA ladder (Roche, Mannheim, Germany) was used as the molecular size standard. The process was run at 120 V for about 45 min, after which the gel was carefully removed from the gel tray and placed in ethidium bromide solution (1 µg/ml) for 30 min and finally was placed on an ultra violet transilluminator to visualize the DNA (Muyzer et al., 1993).

### PCR amplification of 16S-rDNA gene

16S rDNA was amplified by PCR using universal eubacterial forward primer 16F27 (5'-AGA GTT TGA TCC TGG CTC AG-3') and reverse primer 16R1525 (5'-A AG GAG GTG ATC CAG CCG CA-3') derived from *E. coli* 16S-rDNA sequence synthesized by MWG Biotech AG (Ebersberg, Germany). The PCR amplification was performed using the purified genomic DNA as a template. The total volume of the PCR reaction was 50 µL (Forward primer, 1 µ (10 pmol/µL), reverse primer, 1 µ (10 pmol/µL), deoxynucleoside triphosphates 0.6 µL (10 mmol/L), 1 × PCR buffer 3 µL (10 ×), MgCl 21.8 µL (25 mM), Taq polymerase 0.2 µL (5 U/µL), DNA template 2.5 µL (100 ng) and sterile aqua dest water (19.9 µL). The PCR reaction was performed in a Peltier thermal cycler (BioRad, USA) using the following conditions: Initial denaturation 95 °C for 5 min,

(Denaturation 95 °C for 30 second, Annealing 52 °C for 30 second, Extension 70 °C for 1.5 min, final extension 70 °C for 5 min) 34 cycles) and End/store Forever 4.

### Agarose gel electrophoresis of the PCR products

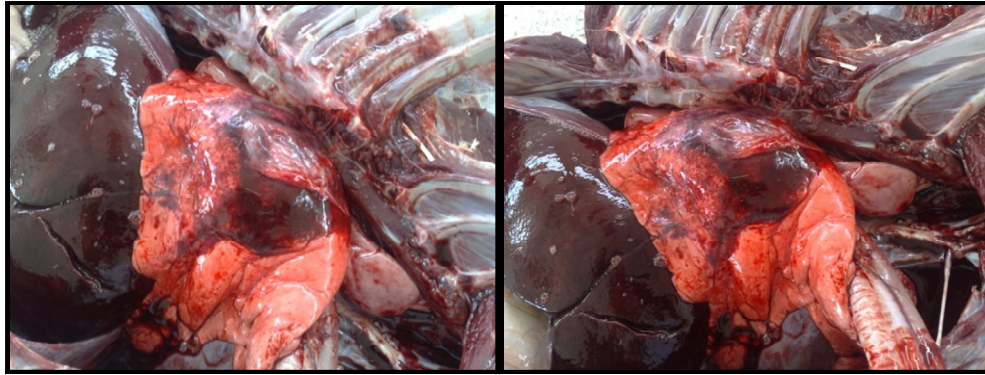
The PCR products (12 µL) were mixed with 3 µL of loading dye solution (Qiagen, NY, USA) and were separated in a 1% (w/v) agarose gel electrophoresis at 120 V in 1 × TAE buffer with a 1 kb DNA ladder (Roche, Mannheim, Germany) as the molecular size standard. The gel was placed in ethidium bromide solution (1 µg/ml) for 30 min and was placed on an ultra violet transilluminator to visualize the DNA (Ausubel et al., 2008). To extract the PCR product from the agarose gel, the gel was washed twice with sterile distilled water and placed on an ultra violet transilluminator to visualize the DNA and the amplified 16S-rDNA products were sliced off from the agarose with sterile razor blade.

The DNA was purified from the agarose using QIAquick gel Extraction Kits. The 16S-rDNA gene of the isolated strains (n = 48) were sent for sequencing to Eurofins MWG Operon Sequencing Facility (Fraunhoferstr, Martinsried, Germany) using the same forward and reverse primers described earlier (Volker et al., 1996). The obtained sequences of 16S-rDNA of the isolates were aligned with the reference 16S-rDNA sequences of the European Microbiological Laboratory (EMBL), GenBank (gb, Germany) and the Database of Japan (DDBJ) that are available in the National Centre for Biotechnology information (NCBI).

## RESULTS

Figure 1 shows health gazelles at the KKWRC, breeding pens. A necrotic cavity containing an air-fluid level was recorded. The infection extended into the pleural space and produced an empyema without rupture of the abscess cavity. The necrotizing pneumonia was restricted to one pulmonary segment or lobe, and in few cases it encompassed an entire lung or both lungs (Figure 2). Microscopic examination of the infected lungs tissues revealed similar suppurative lesions in all of the infected gazelles. Pulmonary changes consisted of multifocal-to-coalescing areas of necrosis, associated with cell debris and fibrin, bounded by low numbers of neutrophils. A direct smear from necrotic areas showed Gram-positive bacilli forming chains (Figure 3). Enrichment and isolation of *A. pyogenes* from the collected samples resulted in the isolation of 48 bacterial strains. The isolates showed colony surrounded with narrow sharp hemolytic zone, morphology similar to those reported for *A. pyogenes* (Figure 4).

Furthermore, cell morphology and size were examined under (SEM) (Figure 5) and Transmission Electron Microscope (TEM) (Figure 6). Cell length and diameter were about 0.8 to 1.0 µm and 0.3 µm, respectively. The bacterial isolates were subjected to further identification by different biochemical tests. API strip identified all isolated bacterial strains (n = 48) as *A. pyogenes*. The results also indicated that all isolates were non-motile, beta-hemolytic on blood agar, able to hydrolyze gelatin and starch, and in addition, the isolates were unable to



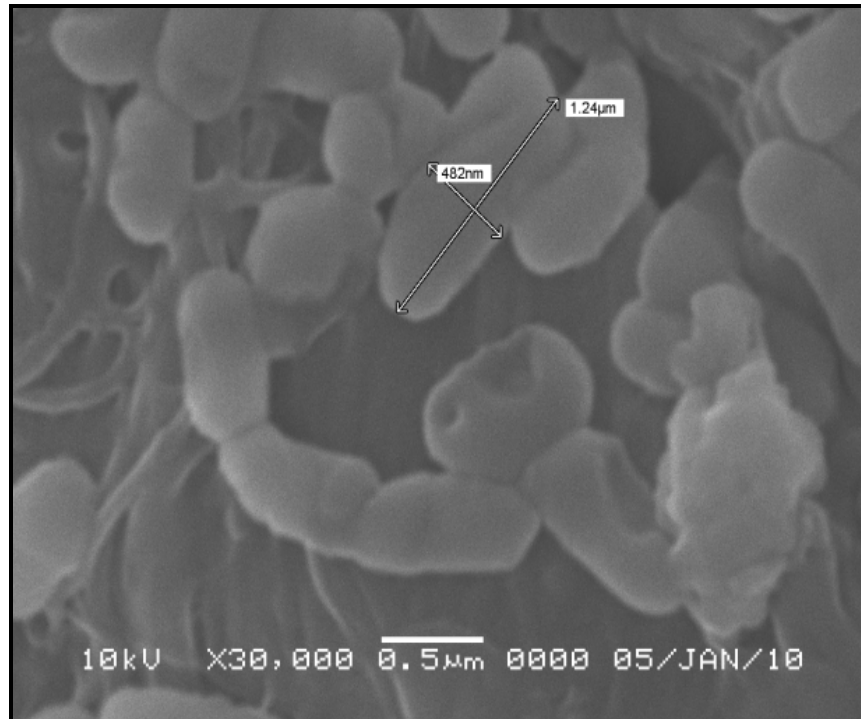
**Figure 1.** Postmortem examination of infected lungs of the gazelle.



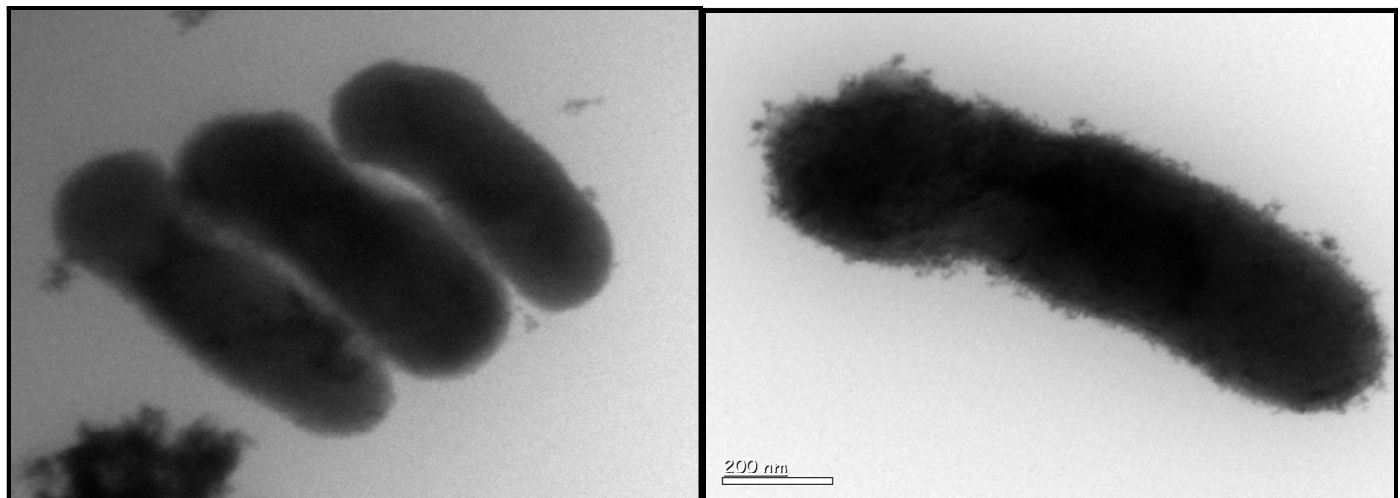
**Figure 2.** Results of the Gram staining of the isolates; isolated strains were gram positive short bacilli.



**Figure 3.** Isolates grown on 5% sheep blood agar medium at 37 °C for 24-72 h. Strains showed colony surrounded with narrow sharp hemolytic zone, morphology similar to those reported for *Actinomyces pyogenes*.



**Figure 4.** Cell morphology of *Actinomyces pyogenes* isolates examined under Scanning Electron Microscope at the Electron Microscopy Unit, National Research Center, Faculty of Science, King Saud University.



**Figure 5.** Cell morphology of *Actinomyces pyogenes* isolates examined under Transmission Electron Microscope (TEM) at the Electron Microscopy Unit, National Research Center, Faculty of Science, King Saud University.

hydrolyze esculin or reduce nitrate and did not produce urease. However, there were considerable variation in other biochemical tests particularly sugars fermentation. The results of the biochemical tests of 11 isolates out of 48 are presented in Table 1.

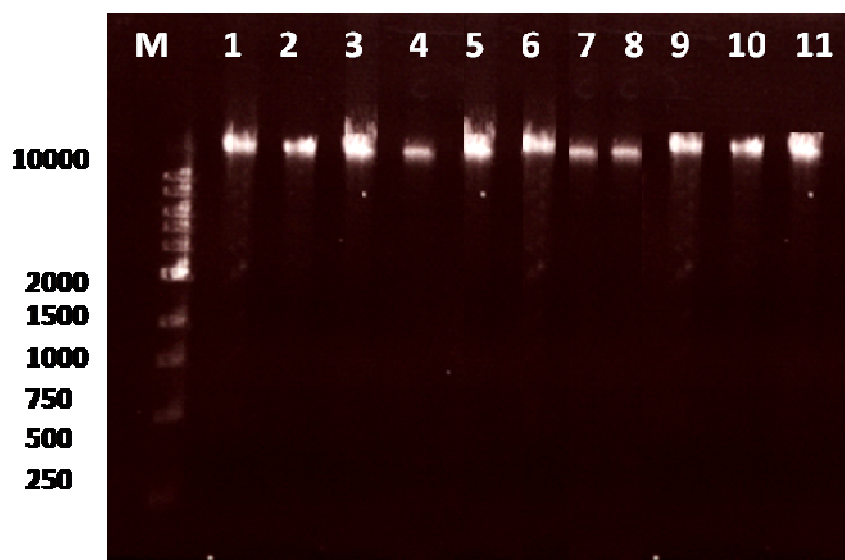
Genomic DNA of the isolated was extracted using DNeasy extraction kit as described earlier. The concen-

tration and purity of the purified bacterial DNA was determined by measuring OD at 260 and 280 nm. Good total DNA yield in the range of 11.7  $\mu\text{g}$  to 27.5  $\mu\text{g}$  was obtained. The DNA purification was repeated for any sample with OD 260/280 ratio higher than 2.0. The extracted DNA was further checked using agarose gel electrophoresis (Figure 7). The amplified 16S rDNA was



**Table 1.** The results of the biochemical tests of 11 isolates out of 48 isolates.

Test	Isolate										
	KGH1	KGH2	KGH3	KGH4	KGH5	KGH6	KGH7	KGH8	KGH9	KGH10	KGH11
β-Hemolysis	+	+	+	+	+	+	+	+	+	+	+
Motility	-	-	-	-	-	-	-	-	-	-	-
Nitrate reduction	-	-	-	-	-	-	-	-	-	-	-
Pyrazinamidase	-	-	-	-	-	-	-	-	-	-	-
Pyrrolidonyl arylamidase	+	+	+	+	+	+	+	+	+	+	+
Alkaline phosphatase	-	-	+	-	+	-	-	+	-	-	-
β-Glucuronidase	+	+	+	+	+	+	+	+	+	+	+
β-Galactosidase	+	+	+	+	+	+	+	+	+	+	+
α-Glucosidase	+	+	+	+	+	+	+	+	+	+	+
N-Acetyl-b- glucosaminidase	+	+	+	+	+	+	+	+	+	+	+
Esculin hydrolysis	-	-	-	-	-	-	-	-	-	-	-
Urease	-	-	-	-	-	-	-	-	-	-	-
Gelatin hydrolysis	+	+	+	+	+	+	+	+	+	+	+
Catalase	-	-	-	-	-	-	-	-	-	-	-
<b>Fermentations of:</b>											
Glucose	+	+	+	+	+	+	+	+	+	+	+
Ribose	+	+	+	+	+	+	+	+	+	+	+
Xylose	+	+	-	+	+	-	+	+	+	-	+
Mannitol	-	-	-	-	-	-	-	-	-	-	-
Maltose	-	+	+	+	-	+	-	+	+	-	+
Lactose	+	+	+	+	+	+	+	+	+	+	+

**Figure 6.** Extracted DNA from *Actinomyces pyogenes* isolates under agarose gel electrophoresis.

sliced off from the agarose and purified from the agarose using QIAquick gel extraction kit with the correct size of about 1525 bp (Figure 8). The 16S rDNA of the isolated strains was amplified by conventional PCR using

universal eubacterial forward primer (16F27) and reverse primer (16R1525), and the PCR products were analyzed using agarose gel electrophoresis and visualized using ultra violet (Figure 9). The sequence of 16S rDNA (length

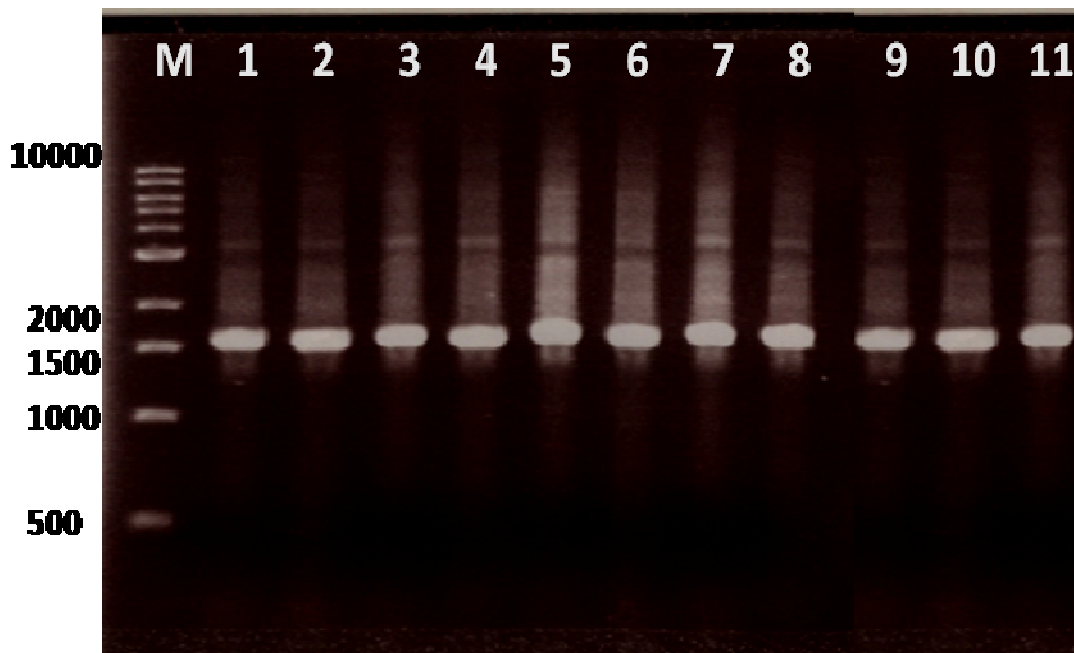


Figure 7. Amplified by 16S rDNA of the isolates by conventional PCR using universal eubacterial forward primer (16F27) and reverse primer (16R1525) under agarose gel electrophoresis

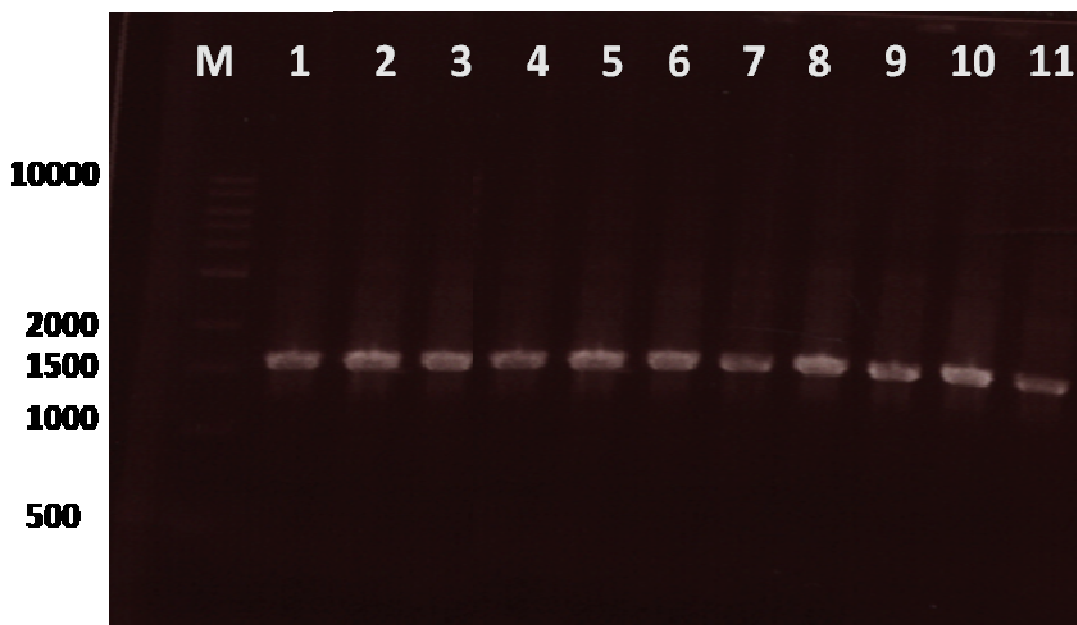


Figure 8. The amplified 16S rDNA sliced off from the agarose gel under electrophoresis.

= 1020 bp) of strain KGD5 has been deposited in the GenBank database with accession number of GU358474 and designed as *A. pyogenes* KGD5 and could be reached using the following website: <http://www.ncbi.nlm.nih.gov/nuccore/283553395> (Figures 10 to 19).

#### DISCUSSION

Many of suppurative lung infections of the captive gazelles have been recorded at KKWRC. *Actinomyces pyogenes* is a common inhabitant of the mucous membranes of the upper respiratory, gastrointestinal, and





```

Query 1   AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAAC 60
          |||
Sbjct 31   AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAAC 90

Query 61   GATGAAGCCGGGGCTTTTGTGTTGGTGGATTAGTGGCGAACGGGTGAGTAATACGTGAG 120
          |||
Sbjct 91   GATGAAGCCGGGGCTTTTGTGTTGGTGGATTAGTGGCGAACGGGTGAGTAATACGTGAG 150

Query 121  TAACCTGCCCTTGTCTTTGGGATAAGCCTGGGAAACTGGGTCTAATACCGGATATTCTGC 180
          |||
Sbjct 151  TAACCTGCCCTTGTCTTTGGGATAAGCCTGGGAAACTGGGTCTAATACCGGATATTCTGC 210

Query 181  TTYGCCGCATGGTGGGGGTGGAAAGATTTTTGGATGGGGATGGGCTCACGGCCTATC 240
          |||
Sbjct 211  TTTTGCCGCATGGTGGGGGTGGAAAGATTTTTGGATGGGGATGGGCTCACGGCCTATC 270

Query 241  AGCTTGTGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGTG 300
          |||
Sbjct 271  AGCTTGTGTTGGTGGGGTGATGGCCTACCAAGGCGTGCACGGGTAGCCGGCCTGAGAGGGTG 330

Query 301  ACCGGCCACATTGGGACTGAGATACGGCCAGACTCCTACGGGAGGCAGCAGTGGGGAA 360
          |||
Sbjct 331  ACCGGCCACATTGGGACTGAGATACGGCCAGACTCCTACGGGAGGCAGCAGTGGGGAA 390

Query 361  ATTGCACAATGGACGCAAGTCTGATGCAGCGACGCCGCGTGGGGGATGAAGGCTTTCGGG 420
          |||
Sbjct 391  ATTGCACAATGGACGCAAGTCTGATGCAGCGACGCCGCGTGGGGGATGAAGGCTTTCGGG 450

Query 421  TTGTAAACTCCTRTCAGTACAGAAGAAGCATTTTTTGTGACGGTATGTGCAGAAGAAGCG 480
          |||
Sbjct 451  TTGTAAACTCCTTTCAGTACAGAAGAAGCA-TTTTGTGACGGTATGTGCAGAAGAAGCG 509

Query 481  CCGGCTAACTACGTGCYAGCAGCCGCGTAATACGTAGGGCGCGAGCGTTGTCCGGAAT 540
          |||
Sbjct 510  CCGGCTAACTACGTGCCAGCAGCCGCGTAATACGTAGGGCGCGAGCGTTGTCCGGAAT 569

Query 541  ATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTGTCGCTGCTGTGAAAGACCGGGGCTTAA 600
          |||
Sbjct 570  ATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTGTCGCTGCTGTGAAAGACCGGGGCTTAA 629

Query 601  CTTCCGGGTTGCAGTGGGTACGGGCAGACTAGAGTGTGGTAGGGTAATTGGAATTCCTG 660
          |||
Sbjct 630  CTTCCGGGTTGCAGTGGGTACGGGCAGACTAGAGTGTGGTAGGGTAATTGGAATTCCTG 689

Query 661  GTGTAGCGGTGGAATGCCAGATATCAGGAGGAACACCGATGGCGAAGGCAGGTTACTGG 720
          |||
Sbjct 690  GTGTAGCGGTGGAATGCCAGATATCAGGAGGAACACCGATGGCGAAGGCAGGTTACTGG 749

Query 721  GCCATTACTGACGCTGAGGAGCGATAGCGTGGGTAGCGAACAGGATTAGATACCCTGGTA 780
          |||
Sbjct 750  GCCATTACTGACGCTGAGGAGCGAAAAGCGTGGGTAGCGAACAGGATTAGATACCCTGGTA 809

Query 781  GTCCACGCTGTAAACGTTGGGCACTAGGTGTGGGGCCTTTCCACGGGTTCTGCGCCGTA 840
          |||
Sbjct 810  GTCCACGCTGTAAACGTTGGGCACTAGGTGTGGGGCCTTTCCACGGGTTCTGCGCCGTA 869

Query 841  GCTAACGCTTTAAGTGCCCCGCTGGGGAGTACGGCCGCAAGGTTAAAACCTCAAAGGAAT 900
          |||
Sbjct 870  GCTAACGCTTTAAGTGCCCCGCTGGGGAGTACGGCCGCAAGGTTAAAACCTCAAAGGAAT 929

Query 901  TGACGGGGGCCCGCACAAAGCGGCGGAGCATGCGGATTAATTCGATG 946
          |||
Sbjct 930  TGACGGGGGCCCGCACAAAGCGGCGGAGCATGCGGATTAATTCGATG 975

```

**Figure 10.** Alignment of 16S rDNA sequence of KGH2 isolate. Identities = 940/946 (99%) with *Arcanobacterium pyogenes* isolate BM-H 07-1 16S ribosomal RNA gene, strand=Plus/Plus. Yellow color: KGH2 sequence.

```

Query 1   AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAAC 60
          |||
Sbjct 21   AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAAC 80

Query 61   GATGAAGCCGGGGCTTTTGTGTTTGGTGGATTAGTGGCGAACGGGTGAGTAATACCTGAG 120
          |||
Sbjct 81   GATGAAGCCGGGGCTTTTGTGTTTGGTGGATTAGTGGCGAACGGGTGAGTAATACCTGAG 140

Query 121  TAACCTGCCCTTGTCTTTGGGATAAGCCTGGGAAACTGGGTCTAATACCGGATATTCTGC 180
          |||
Sbjct 141  TAACCTGCCCTTGTCTTTGGGATAAGCCTGGGAAACTGGGTCTAATACCGGATATTCTGC 200

Query 181  TTTTGCCGCATGGTGGGGGTGGAAAGATTTTTGGATGGGGATGGGCTCACGGCCTATC 240
          |||
Sbjct 201  TTTTGCCGCATGGTGGGGGTGGAAAGATTTTTGGATGGGGATGGGCTCACGGCCTATC 260

Query 241  AGCTTGTGGTGGGGTGATGGCCTACCAAGGCGTCGACGGGTAGCCGCTCTGAGAGGGTG 300
          |||
Sbjct 261  AGCTTGTGGTGGGGTGATGGCCTACCAAGGCGTCGACGGGTAGCCGCTCTGAGAGGGTG 320

Query 301  ACCGGCCACATTGGGACTGAGATACGGCCAGACTCCTACGGGAGGCAGCAGTGGGGAAT 360
          |||
Sbjct 321  ACCGGCCACATTGGGACTGAGATACGGCCAAACTCCTACGGGAGGCAGCAGTGGGGAAT 380

Query 361  ATTGCACAATGGACGCAAGTCTGATGCAGCGACGCCGCGTGGGGATGAAGGCTTTCGGG 420
          |||
Sbjct 381  ATTGCACAATGGACGCAAGTCTGATGCAGCGACGCCGCGTGGGGATGAAGGCTTTCGGG 440

Query 421  TTGTAAACTCCTTTCAGTACAGAAGAAGCATTCTTGTGACGGTATGTGCAGAAGAAGCG 480
          |||
Sbjct 441  TTGTAAACTCCTTTCAGTACAGAAGAAGCATTCTTGTGACGGTATGTGCAGAAGAAGCG 499

Query 481  CCGGCTAACTACGTGCCAGCAGCCGCGTAATACGTAGGGCGCGAGCGTTGTCCGGAATT 540
          |||
Sbjct 500  CCGGCTAACTACGTGCCAGCAGCCGCGTAATACGTAGGGCGCGGGCGTTGTCCGGAATT 559

Query 541  ATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTGCGCCTGCTGTGAAAGACCGGGCTTAA 600
          |||
Sbjct 560  ATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTGCGCCTGCTGTGAAAGACCGGGCTTAA 619

Query 601  CTTGGGGTTGCACTGGGTACGGGCAGACTAGCGTGTGGTAGGGGTAATTGGAATTCCTG 660
          |||
Sbjct 620  CTTGGGGTTGCACTGGGTACGGGCAGACTAGAGTGTGGTAGGGGTAATTGGAATTCCTG 679

Query 661  GTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGGTTACTGG 720
          |||
Sbjct 680  GTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGGTTACTGG 739

Query 721  GCCATTACTGACGCTGAGGAGCGAAAGCGTGGGTAGCGAACAGGATTAGATACCCTGGTA 780
          |||
Sbjct 740  GCCATTACTGACGCTGAGGAGCGAAAGCGTGGGTAGCGAACAGGATTAGATACCCTGGTA 799

Query 781  GTCCACGCTGTAAACGTTGGGCACTAGGTGTGGGGCCTTTTCCACGGGTTCTGCGCCGTA 840
          |||
Sbjct 800  GTCCACGCTGTAAACGTTGGGCACTAGGTGTGGGGCCTTTTCCACGGGTTCTGCGCCGTA 859

Query 841  GCTAACGCTTTAAGTGCCCCGCCTGGGGAGTACGG 875
          |||
Sbjct 860  GCTAACGCTTTAAGTGCCCCGCCTGGGGAGTACGG 894

```

**Figure 11.** Alignment of 16S rDNA sequence of KGH3 isolate. Identities = 869/875 (99%) with *Arcanobacterium pyogenes* isolate HC-H 10 16S ribosomal RNA gene, strand=Plus/Plus. Yellow color: KGH3 sequence.

```

Query 1 AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTTGAAC 60
      |||
Sbjct 31 AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAAC 90

Query 61 GATGAAGCCGGGGCTTTTGTGTTTGGTGGATTAGTGGCGAACGGGTGAGTAATACGTGAG 120
      |||
Sbjct 91 GATGAAGCCGGGGCTTTTGTGTTTGGTGGATTAGTGGCGAACGGGTGAGTAATACGTGAG 150

Query 121 TAACCTGCCCTTGTCTTTGGGATAAGCCTGGGAAACTGGGTCTAATACCGGATATTCTGC 180
      |||
Sbjct 151 TAACCTGCCCTTGTCTTTGGGATAAGCCTGGGAAACTGGGTCTAATACCGGATATTCTGC 210

Query 181 TTTTGCCGCATGGTGGGGTTGGAAAGATTTTTGGATGGGGATGGGCTCACGGCCTATC 240
      |||
Sbjct 211 TTTTGCCGCATGGTGGGGTTGGAAAGATTTTTGGATGGGGATGGGCTCACGGCCTATC 270

Query 241 AGCTTGTGGTGGGGTGATGGCCTACCAAGGCGTCGACGGGTAGCCGGCCTGACAGGGTG 300
      |||
Sbjct 271 AGCTTGTGGTGGGGTGATGGCCTACCAAGGCGTCGACGGGTAGCCGGCCTGACAGGGTG 330

Query 301 ACCGGCCACATTGGGACTGAGATACGGCCAGACTCCTACGGGAGGCAGCAGTGGGGAAT 360
      |||
Sbjct 331 ACCGGCCACATTGGGACTGAGATACGGCCAGACTCCTACGGGAGGCAGCAGTGGGGAAT 390

Query 361 ATTGCACAATGGACGCAAGTCTGATGCAGCGACGCCGCTGGGGTTGAAGGCTTTCGGG 420
      |||
Sbjct 391 ATTGCACAATGGACGCAAGTCTGATGCAGCGACGCCGCTGGGGTTGAAGGCTTTCGGG 450

Query 421 TTGTAAACTCCTTTCAGTACAGAAGAAGCATTTRTTGTGACGGTATGTGCAGAAGAAGCG 480
      |||
Sbjct 451 TTGTAAACTCCTTTCAGTACAGAAGAAGCATT- TTTGTGACGGTATGTGCAGAAGAAGCG 509

Query 481 CCGGCTAACTACGTGCCAGCAGCCGCGTAATACGTAGGGCGCGAGCGTTGTCCGGAATT 540
      |||
Sbjct 510 CCGGCTAACTACGTGCCAGCAGCCGCGTAATACGTAGGGCGCGAGCGTTGTCCGGAATT 569

Query 541 ATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTTCGCCTGCTGTGAAAGACCGGGCTTAA 600
      |||
Sbjct 570 ATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTTCGCCTGCTGTGAAAGACCGGGCTTAA 629

Query 601 CTTCGGGTTGCAGTGGGTACGGGCAGACTAGAGTGTGGTAGGGGTAATTGGAATTCCTG 660
      |||
Sbjct 630 CTTCGGGTTGCAGTGGGTACGGGCAGACTAGAGTGTGGTAGGGGTAATTGGAATTCCTG 689

Query 661 GTGTAGCGGTGGAATGCGCAGATATCABGAGGAACACCGATGGCGAAGGCAGGTTACTGG 720
      |||
Sbjct 690 GTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGGTTACTGG 749

Query 721 GCCATTACTGACGCTGAGGAGCGAAAGCGTCGGTAGCGAACAGGATTAGAT 771
      |||
Sbjct 750 GCCATTACTGACGCTGAGGAGCGAAAGCGTGGGTAGCGAACAGGATTAGAT

```

**Figure 12.** Alignment of 16S rDNA sequence of KGD4 isolate. Identities = 763/771 (98%) with *Arcanobacterium pyogenes* isolate BM-H 07-1 16S ribosomal RNA gene, strand=Plus/Plus. **Yellow color:** of KGD4 sequence.

Query	1	AGAGTTTGATCCTCGCTCAGGACGAACGCTGGCGGCGTGCCTAACACATGCAAGTCGAAC	60
Sbjct	24	AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAACACATGCAAGTCGAAC	83
Query	61	GATGAAGCCGGGGCTTTTGTGTTGGTGGAATTAGTGGCGAACGGGTGAGTAATACGTGAG	120
Sbjct	84	GATGAAGCCGGGGCTTTTGTGTTGGTGGAATTAGTGGCGAACGGGTGAGTAATACGTGAG	143
Query	121	TAACCTGCCCTTGTCTTTGGGATAAGCCTGGGAACTGGGTCTAATACCGGATATTCTGC	180
Sbjct	144	TAACCTGCCCTTGTCTTTGGGATAAGCCTGGGAACTGGGTCTAATACCGGATATTCTGC	203
Query	181	CTTTGCCGCATGGTGGGGGTGGAAAAGATTTTTGGATGGGGATGGGCTCACGGCCTATC	240
Sbjct	204	CTTTGCCGCATGGTGGGGGTGGAAAAGATTTTTGGATGGGGATGGGCTCACGGCCTATC	263
Query	241	AGCTTGTGGTGGGGTGATGGCCTACCAAGCGTCGACGGGTAGCCGGCTGAGAGGGTG	300
Sbjct	264	AGCTTGTGGTGGGGTGATGGCCTACCAAGCGTCGACGGGTAGCCGGCTGAGAGGGTG	323
Query	301	ACCGGCCACATGGGACTGAGATACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAAI	360
Sbjct	324	ACCGGCCACATGGGACTGAGATACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAAI	383
Query	361	ATTGCACAATGGACGCAAGTCTGATGCAGCGACGCCGCGTGGGGGATGAAGGCTTTCGGG	420
Sbjct	384	ATTGCACAATGGACGCAAGTCTGATGCAGCGACGCCGCGTGGGGGATGAAGGCTTTCGGG	443
Query	421	TTGTAAACTCCTTTCAGTACAGAAGAAGCATTTTTTGTGACGGTATGTGCAGAAGAAGCG	480
Sbjct	444	TTGTAAACTCCTTTCAGTACAGAAGAAGCATTTTTTGTGACGGTATGTGCAGAAGAAGCG	503
Query	481	CCGGCTAACTACGTGCCAGCAGCCGGTAATACGTAGGGCGGAGCGTTGTCCGGAATT	540
Sbjct	504	CCGGCTAACTACGTGCCAGCAGCCGGTAATACGTAGGGCGGAGCGTTGTCCGGAATT	563
Query	541	ATTGGCGTAAAGAGCTCGTAGCCGTTTGTGTRGCCGTGTGAAAGACCGGGGCTTAA	600
Sbjct	564	ATTGGCGTAAAGAGCTCGTAGCCGTTTGTGTRGCCGTGTGAAAGACCGGGGCTTAA	623
Query	601	CTTCGGGGTTGCAGTGGGTACGGGCAGACTAGAGTGTGGTAGGGGTAATTGGAATTCCTG	660
Sbjct	624	CTTCGGGGTTGCAGTGGGTACGGGCAGACTAGAGTGTGGTAGGGGTAATTGGAATTCCTG	683
Query	661	GTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGGTTACTGG	720
Sbjct	684	GTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGGTTACTGG	743
Query	721	GCCATTACTGACGCTGAGGAGCGAAAAGCGTGGGTAGCGAACAGGATTAGATACCCTGGTA	780
Sbjct	744	GCCATTACTGACGCTGAGGAGCGAAAAGCGTGGGTAGCGAACAGGATTAGATACCCTGGTA	803
Query	781	GTCCACGCTGTAACGTTGGGCACTAGGTGTGGGGCCTTTCCACGGGTCTGCGCCGTA	840
Sbjct	804	GTCCACGCTGTAACGTTGGGCACTAGGTGTGGGGCCTTTCCACGGGTCTGCGCCGTA	863
Query	841	GCTAACGCTTTAAGTGCCCCGCTGGGGAGTACGGCCGCAAGGTTAAACTCAAAGGAAT	900
Sbjct	864	GCTAACGCTTTAAGTGCCCCGCTGGGGAGTACGGCCGCAAGGTTAAACTCAAAGGAAT	923
Query	901	TGACGGGGGCCCCGACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACCGGAAGAACC	960
Sbjct	924	TGACGGGGGCCCCGACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACCGGAAGAACC	983
Query	961	TTACCAAGGMTGACATACACTGCGATGTGCCAGAGTGGTGCAGCCTTCGGGGTGGTGT	1020
Sbjct	984	TTACCAAGGCTTGACATACACTGCGATGTGCCAGAGATGGTGCAGCCTTCGGGGTGGTGT	1043

**Figure 13.** Alignment of 16S rDNA sequence of KGD5 isolate. Identities = 1015/1020 (99%) with *Arcanobacterium pyogenes* HC-H 10 16S ribosomal RNA gene, strand=Plus/Plus. **Yellow color:** KGD5 sequence.

```

Query 1   AGAGTTTGATCCTGGCTCAGGACGAACGYTGGCGGCGTGCTTAACACATGCAAGTCGAAC 60
          |||
Sbjct 24   AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAAC 83

Query 61  YATGAAGCCGGGGCTTTTTGTTTTGGTGGATTAGTGGCGAACGGGTGAGTAATACGTGAG 120
          |||
Sbjct 84   GATGAAGCCGGGGCTTTTTGTTTTGGTGGATTAGTGGCGAACGGGTGAGTAATACGTGAG 143

Query 121 TAACCTGCCCTTGCTTTGGGATAAGCCTGGGAACTGGGTCTAATACCGGATATTCTGC 180
          |||
Sbjct 144  TAACCTGCCCTTGCTTTGGGATAAGCCTGGGAACTGGGTCTAATACCGGATATTCTGC 203

Query 181  TTTGCCGCATGGTGGGGGTGGAAAGATTTBTGGATGGGGATGGGCTCACGGCCTATC 240
          |||
Sbjct 204  TTTGCCGCATGGTGGGGGTGGAAAGATTTTTGGATGGGGATGGGCTCACGGCCTATC 263

Query 241  AGCTTGTGGTGGGGTGATGGCCTACCAAGGCGTCGACGGGTRGCCGGCCTGAGAGGGTG 300
          |||
Sbjct 264  AGCTTGTGGTGGGGTGATGGCCTACCAAGGCGTCGACGGGTRGCCGGCCTGAGAGGGTG 323

Query 301  ACCGGCCACATTGGGACTGAGATACGGCCAGACTCCTACGGGAGGCAGCAGTGGGGAAT 360
          |||
Sbjct 324  ACCGGCCACATTGGGACTGAGATACGGCCAGACTCCTACGGGAGGCAGCAGTGGGGAAT 383

Query 361  ATTGCACAATGGACGCAAGTCTGATGCAGCGACGCCGCTGGGGGATGAAGGCTTTCGGG 420
          |||
Sbjct 384  ATTGCACAATGGACGCAAGTCTGATGCAGCGACGCCGCTGGGGGATGAAGGCTTTCGGG 443

Query 421  TTGTAACTCCTTTCAGTACAGAAGAAYCATTTTTTGTGACGGTATGTGCAGAAGAAGCG 480
          |||
Sbjct 444  TTGTAACTCCTTTCAGTACAGAAGAAGCATTTTTTGTGACGGTATGTGCAGAAGAAGCG 503

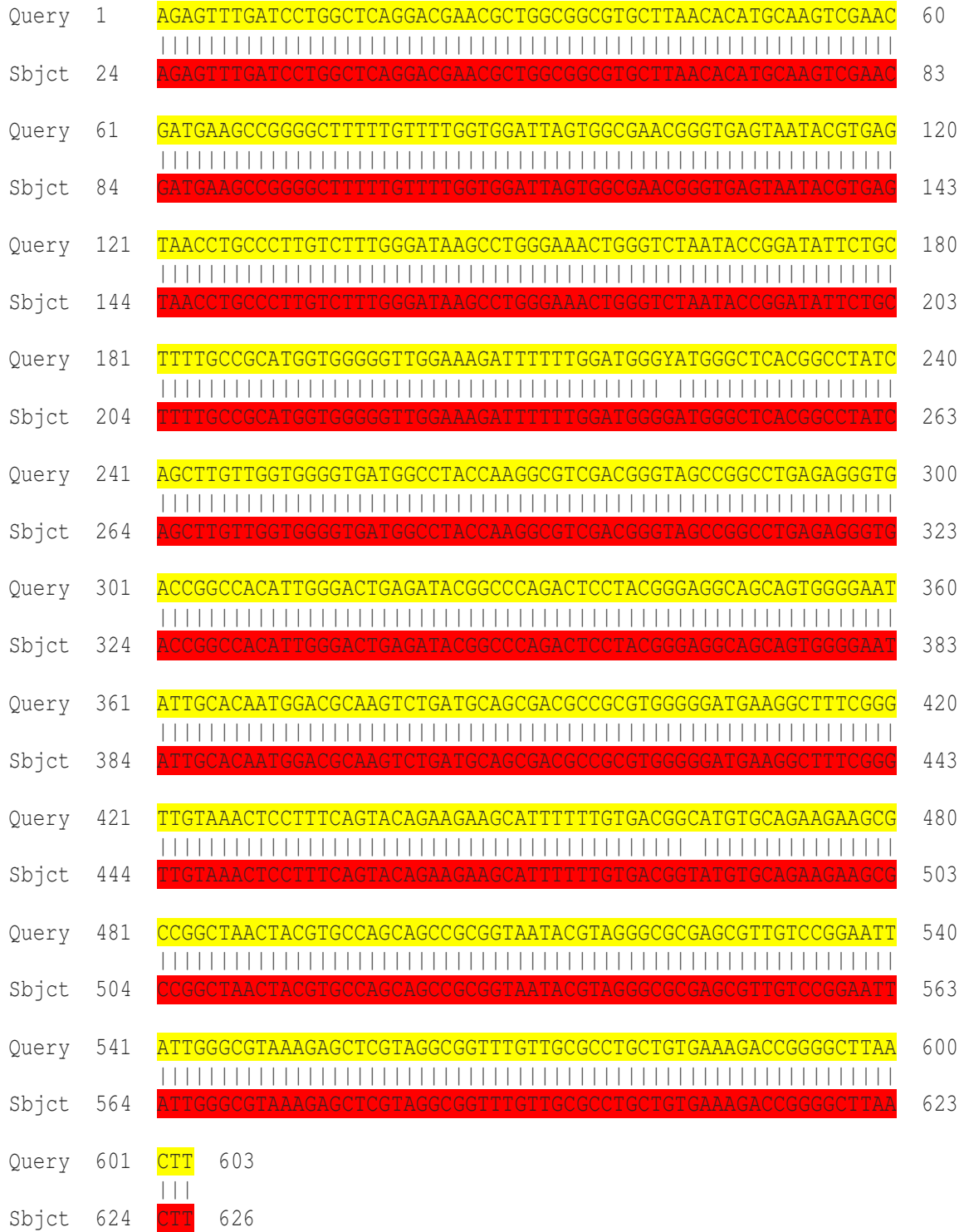
Query 481  CCGGCTAACTACGTGCCAGCAGCCGCGTAATACGTAGGGCRGAGCGTTGTCCGGAATT 540
          |||
Sbjct 504  CCGGCTAACTACGTGCCAGCAGCCGCGTAATACGTAGGGCRGAGCGTTGTCCGGAATT 563

Query 541  ATTGGCGTAAAGAGCTCGTAGGCGGTTTGTTCGCCTGCTGTGAAAGACCGGGGCTTAA 600
          |||
Sbjct 564  ATTGGCGTAAAGAGCTCGTAGGCGGTTTGTTCGCCTGCTGTGAAAGACCGGGGCTTAA 623

Query 601  CTTCGGGGTTGCAGTGGGTACGGGCAG 627
          |||
Sbjct 624  CTTCGGGGTTGCAGTGGGTACGGGCAG 650

```

**Figure 14.** Alignment of 16S rDNA sequence of KGD6 isolate, Identities = 621/627 (99%) *Arcanobacterium pyogenes* isolate HC-H 10 16S ribosomal RNA gene, strand=Plus/Plus. **Yellow color**: KGD6 sequence.



**Figure 16.** Alignment of 16S rDNA sequence of KGD8 isolate. Identities = 601/603 (99%) with *Arcanobacterium pyogenes* isolate HC-H 16S ribosomal RNA gene, strand=Plus/Plus. **Yellow color:** KGD8 sequence.

urogenital tracts of a number of domestic animal species (Jost et al, 2005). The objectives of this study were to investigate the epidemiology of *A. pyogenes* in the infected gazelles under captive at King Khalid Wildlife

Research Center, investigation of the relationship between symptoms and infection by *A. Pyogenes*. Cell morphology and size were examined under SEM and TEM. Cells were about 0.8-1.0 µm and 0.3 µm in length



```

Query 1   AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAAC 60
          |||
Sbjct 24   AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAAC 83

Query 61   GATGAAGCCGGGGCTTTTTGTTTTGGTGGATTAGTGGCGAACGGGAGAGTAATACGTGAG 120
          |||
Sbjct 84   GATGAAGCCGGGGCTTTTTGTTTTGGTGGATTAGTGGCGAACGGGTGAGTAATACGTGAG 143

Query 121  TAACCTGCCCTTGTCTTTGGGATARGCCTGGGAAACTGGGTCTAATACCGGATATTCTGC 180
          |||
Sbjct 144  TAACCTGCCCTTGTCTTTGGGATAAGCCTGGGAAACTGGGTCTAATACCGGATATTCTGC 203

Query 181  TTTTGCCGCATGGTGTGGGTGGAAAGATTTTTGGATGGGGATGGGCTCACGGCCTATC 240
          |||
Sbjct 204  TTTTGCCGCATGGTGGGGGTGGAAAGATTTTTGGATGGGGATGGGCTCACGGCCTATC 263

Query 241  AGCTTGTGGTGGGGTGATGGCCTACCAAGGCGTCGACGGGTAGCCGGCTGAGAGGGTG 300
          |||
Sbjct 264  AGCTTGTGGTGGGGTGATGGCCTACCAAGGCGTCGACGGGTAGCCGGCTGAGAGGGTG 323

Query 301  ACCGGCCACATTGGGACTGAGATACGGCCAGACTCCTACGGGAGGCAGCAGTGGGGAAT 360
          |||
Sbjct 324  ACCGGCCACATTGGGACTGAGATACGGCCAGACTCCTACGGGAGGCAGCAGTGGGGAAT 383

Query 361  ATTGWACAATGGACGCAAYTCTGATGCAGCGACGCCGCGTGGGGGATGAAGGCTTTCGGG 420
          |||
Sbjct 384  ATTGCACAATGGACGCAAGTCTGATGCAGCGACGCCGCGTGGGGGATGAAGGCTTTCGGG 443

Query 421  TTGTAAACTCCTTTCAGTACAGAAGAAGCATTTTTTGTGACGGTATGTGCAGBAGAAGCG 480
          |||
Sbjct 444  TTGTAAACTCCTTTCAGTACAGAAGAAGCATTTTTTGTGACGGTATGTGCAGAGAAGCG 503

Query 481  CCGGCTAACTACGTGCCAGCAGCCGCGTAATACGTAGGGCGCGAGCGTTGTCCGGAAYT 540
          |||
Sbjct 504  CCGGCTAACTACGTGCCAGCAGCCGCGTAATACGTAGGGCGCGAGCGTTGTCCGGAATT 563

Query 541  ATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTGTGCGCCTGCTGTGAAAGACCGGGGCTTAA 600
          |||
Sbjct 564  ATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTGTGCGCCTGCTGTGAAAGACCGGGGCTTAA 623

Query 601  CTTGGGGTTGCAGTGGGTACGGGAGACTAGAGTGTGGTAGGGGTAATTGGAATTCCTG 660
          |||
Sbjct 624  CTTGGGGTTGCAGTGGGTACGGGAGACTAGAGTGTGGTAGGGGTAATTGGAATTCCTG 683

Query 661  GTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGGTTACTGG 720
          |||
Sbjct 684  GTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGGTTACTGG 743

Query 721  GCCATTACTGACGCTGABGAGCGAAAGCGTGGGTAGCGAACAGGATTAGATACCCTGGTA 780
          |||
Sbjct 744  GCCATTACTGACGCTGAGGAGCGAAAGCGTGGGTAGCGAACAGGATTAGATACCCTGGTA 803

Query 781  GTC 783
          |||
Sbjct 804  GTC 806

```

**Figure 17.** Alignment of 16S rDNA sequence of KGD9 isolate, Identities = 774/783 (98%) with *Arcanobacterium pyogenes* isolate HC-H 10 16S ribosomal RNA gene, strand=Plus/Plus. **Yellow color:** KGD9 sequence.

and diameter respectively. The isolated bacterial strains (n = 48) were subjected to identification by biochemical testes and API Coryne strip. The biochemical tests

revealed that all isolates were beta-hemolytic on blood agar, able to hydrolyze gelatin and starch, in addition, the isolates were unable to hydrolyze esculin or reduce

```

Query 1   AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAAC 60
          |||
Sbjct 24   AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAAC 83

Query 61   GATGAAGCCGGGGCTTTTIGTTTTGGTGGATTAGTGGCGAACGGGTGAWTAATACGTGAG 120
          |||
Sbjct 84   GATGAAGCCGGGGCTTTTIGTTTTGGTGGATTAGTGGCGAACGGGTGAGTAATACGTGAG 143

Query 121  TAACCTGCCCTTGTCTTTGGGATAAGCCTGGGAAACTGGGTCTAATACCGGATATTCTGC 180
          |||
Sbjct 144  TAACCTGCCCTTGTCTTTGGGATAAGCCTGGGAAACTGGGTCTAATACCGGATATTCTGC 203

Query 181  TTTTGCCGCATGGTGGGGGTGGAAAGATTTTTTGGATGGGGATGGGCTCACGGCCTATC 240
          |||
Sbjct 204  TTTTGCCGCATGGTGGGGGTGGAAAGATTTTTTGGATGGGGATGGGCTCACGGCCTATC 263

Query 241  AGCTTGTGGTGGGGTGTATGGCCTACCAAGGCGTCGACGGGTAGCCGGCCTGAGAGGGTG 300
          |||
Sbjct 264  AGCTTGTGGTGGGGTGTATGGCCTACCAAGGCGTCGACGGGTAGCCGGCCTGAGAGGGTG 323

Query 301  ACCGGCCACATTGGGACTGACATACGGCCAGACTCBTACGGGAGGCAGCAGTGGGGAAAT 360
          |||
Sbjct 324  ACCGGCCACATTGGGACTGAGATACGGCCAGACTCCTACGGGAGGCAGCAGTGGGGAAAT 383

Query 361  ATTGCACAATGGACBCAAGTCTGATGCAGCGACGCCGCTGGGGGATGAAGGCTTTCGGG 420
          |||
Sbjct 384  ATTGCACAATGGACBCAAGTCTGATGCAGCGACGCCGCTGGGGGATGAAGGCTTTCGGG 443

Query 421  TTGTAAACTCCTTTCGGTACAGAAGAAGCATTTT--GTGACGGTATGTGCAGATGAAGCG 478
          |||
Sbjct 444  TTGTAAACTCCTTTCAGTACAGAAGAAGCATTTTTTGTGACGGTATGTGCAGAGAAGCG 503

Query 479  CCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCGAGCGTTGTCCGGAATT 538
          |||
Sbjct 504  CCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCGAGCGTTGTCCGGAATT 563

Query 539  ATTGGGCGTAAAGAGCTCGTAGGCGRTTGTGTGCGCCTGCTGTGAAAGACCGGGGCTTAA 598
          |||
Sbjct 564  ATTGGGCGTAAAGAGCTCGTAGGCGRTTGTGTGCGCCTGCTGTGAAAGACCGGGGCTTAA 623

Query 599  CTTCCGGGTTGCAGTGGGTACGGGCAGACTAGAGTGTGGTAGGGGTAATTGGAATTCCTG 658
          |||
Sbjct 624  CTTCCGGGTTGCAGTGGGTACGGGCAGACTAGAGTGTGGTAGGGGTAATTGGAATTCCTG 683

Query 659  GTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGGTTACTGG 718
          |||
Sbjct 684  GTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGGTTACTGG 743

Query 719  GCCATTACTGACGCTGAGGAGCGAAA 744
          |||
Sbjct 744  GCCATTACTGACGCTGAGGAGCGAAA 769

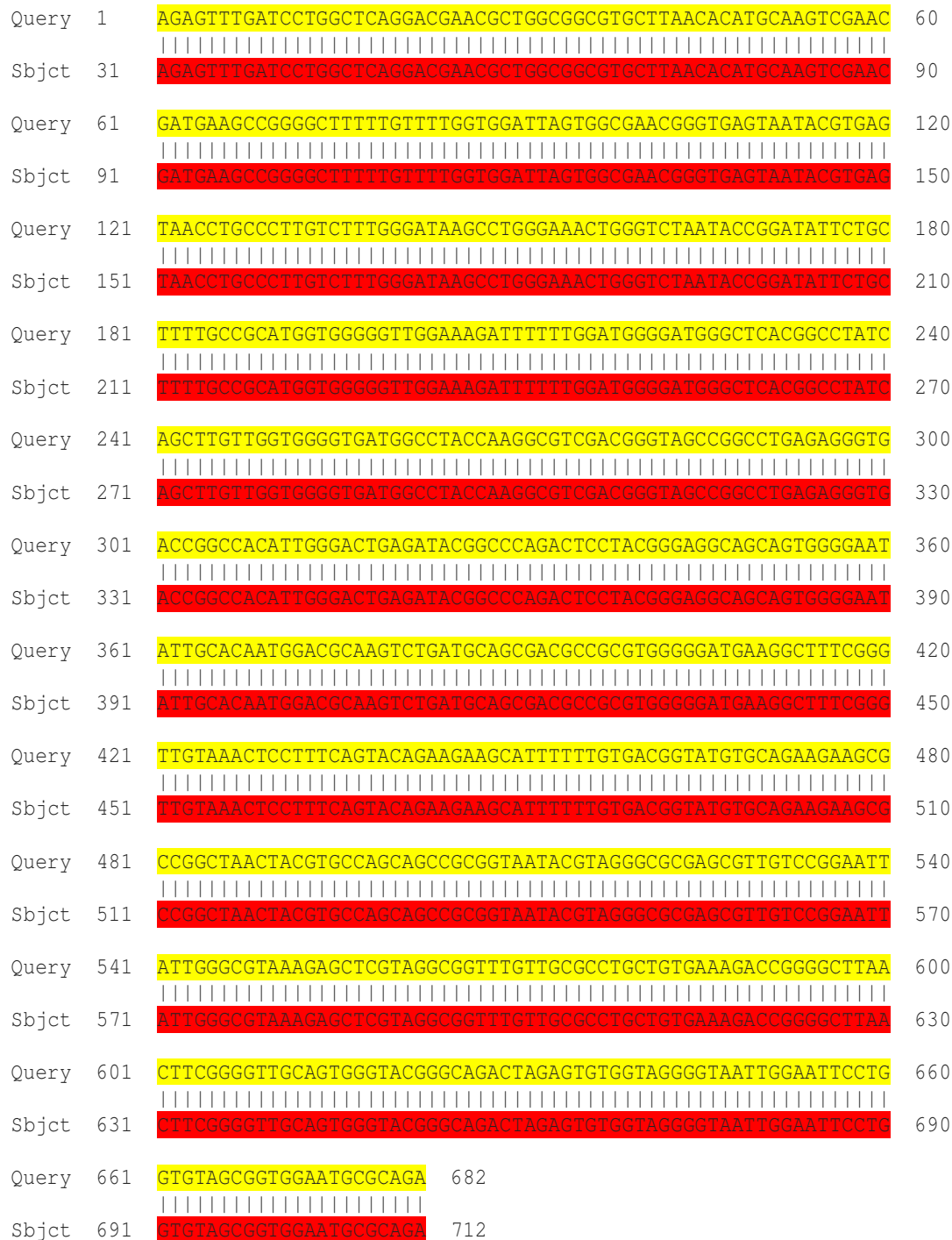
```

**Figure 18.** Alignment of 16S rDNA sequence of KGD10 isolate. *Arcanobacterium pyogenes* isolate HC-H 10 16S ribosomal RNA gene, Identities = 737/746 (98%), strand=Plus/Plus. **Yellow color:** KGD10 sequence.

nitrate and did not produce urease which are typical characteristics of *A. pyogenes* according to Bergey's Manual of Systematic Bacteriology (Farmer III, et al., 2005). However, there was some variation in sugar fermentation ability of different isolates which are also similar to the results reported for *A. pyogenes* by other scientists who reported that different strains of *A. pyogenes* showed variable fermentation ability of sugars

(Jost et al, 2005).

The results of the bacterial isolation of samples collected from pleural fluid of infected gazelles indicated that *A. pyogenes* was detected in 57% of the infected gazelles (40 out of 48 pleural fluid samples). The identification of the isolated strains was further confirmed by molecular method using analysis of 16S rDNA gene sequencing. The PCR products were analyzed using



**Figure 19.** Alignment of 16S rDNA sequence of KGD11 isolate. *Arcanobacterium pyogenes* isolate HC-H 11 16S ribosomal RNA gene.

agarose gel electrophoresis and purified from the agarose gel. Purified 16S-rDNA fragment was sequenced twice and were obtained up to 1020 bases with minimal mistakes. The 16S-rDNA sequences of the isolated bacterial strains were compared and aligned with the known 16S-rDNA sequences of other bacteria available

in the GenBank database. It was found that the isolated bacterial strains had 98% to 100% similarity with different strains of *A. pyogenes*. It has been reported that 16S-rDNA gene sequences similarity of  $\geq 97\%$  indicated the same bacterial species (Muyzer et al., 1993). Therefore, the isolated strains; to best of our knowledge are the first

report of *A. pyogenes* isolation from infected Arabian gazelles in Saudi Arabia.

## ACKNOWLEDGEMENT

We would like to express our sincere gratitude and deep gratefulness to King Saud University RGP-VPP-137 for their financial support. We also thank all the staffs of Botany and Microbiology Department for their advice and support.

## REFERENCES

- Bosshard PP, Abels S, Zbinden R, Böttger EC, Altwegg M, (2003). Ribosomal DNA Sequencing for identification of aerobic Gram positive rods in the clin. laboratory (an 18-months evaluation). *J. Clin. Microbiol.* 41: 4134-4140.
- Carlos Emilio Levy, Rogerio Jesus Pedro, Angela Von Nowakonski, Luciana Maria Holanda, Marcelo Brocchi, Marcelo Carvalho Ramo (2009). *Arcanobacterium pyogenes* Sepsis in Farmer, Brazil. *J. of Emerg. Infect. Disea.* 15: 1131-1132.
- Ceribasi AO, Eroksuz Y, Ceribasi S, Ozer H (2009) Generalized AA amyloidosis and fibrino-hemorrhagic pancreatitis in a *Gazelle subgutturosa*: a case report. *Veterin. Med.* , 54: 340-344.
- Clarridge III J E (2004). Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious disease. *Clin. Microbiol. Rev.* 17: 840- 862.
- Ertas HB, kilic A, Ozbey G, Muz A (2003). Isolation of *Arcanobacterium (Actinomyces) pyogenes* from Abscessed Cattle Kidney and Identification by PCR. *Turk J. Vet Anim Sci.* 29: 455-459
- Farmer III JJ, Janda M, Brenner FW, Cameron DN, Birkhead KM (2005). *Bergey's Manual of Systematic Bacteriology*, 2: 494-546.
- Gouletsou PG, Fthenakis GC, Tzora A, Cripps PJ, Saratsi P (2006). Isolation of *Arcanobacterium pyogenes* from the scrotal skin and the prepuce of healthy rams or from rams with testicular abnormalities. *Small Rumin. Res.* 63: 177-182.
- Hermida Ameijeras A, Romero Jung P, Cabarcos Ortiz de Barron A, Treviño Castallo M ( 2004). One case of pneumonia with *Arcanobacterium pyogenes* [in Spanish]. *An Med. Int. (Madrid)*. 21: 334-336.
- Jost BH, Billington SJ (2005). *Arcanobacterium pyogenes*: Molecular pathogenesis of an animal opportunistic pathogens. *Antonie van Leeuwenhoek.* 88: 87-102.
- Liu MC, Wu CM, Liu YC, Zhao JC, Yang YL, Shen JZ (2009). Identification, susceptibility, and detection of integron-gene cassettes of *Arcanobacterium pyogenes* in bovine endometritis. *J. Dairy Sci.* 92: 3659-3666.
- Meghan C, Radcliffe W, Seals RC Lewis GS (2005). Uterine Response to Multiple Inoculations with *Arcanobacterium pyogenes* and *Escherichia coli* in Nulliparous Ewes. *Amer. J. of Reprod. Immun.* 54: 249-26.
- Mignard S, Flandrois JP (2006). 16S rRNA sequencing in routine bacterial identification: a 30-month exp. *J. Microbiol. Methol.* 67: 574-578.
- Muyzer G, de Waal EC, Uitterlinden AG (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis by polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl. Environ. Microbiol.* 59: 695-700.
- Plamondon M, Martinez G, Raynal L, Touchette M, Valiquette L (2007). A fatal case of *Arcanobacterium pyogenes* endocarditis in a man with no identified animal contact: case report and review of the literature. *Eur. J. Clin. Microbiol. Infect Dis.* 26(9): 663-666.
- Tamura K, Dudley J, Nei M, Kumar S (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol.* 24:1596-15969.
- Volker Gurtler, Vilma A. Stanisich (1996). New approaches to typing and identification of bacteria using the 16s-23s rDNA spacer region. *Microbiol.* 142: 3-16.