



# Diagnostic Evaluation of *Streptococcus gallolyticus* Infection in Patients with Colon Diseases by PCR Method

Foroogh Eshaghi<sup>1</sup>, Haniyeh Bashizadeh-Fakhar<sup>2,\*</sup>, Masood Ghane<sup>3</sup> and Javad Shokry<sup>4</sup>

<sup>1</sup>Department of Medical Science, Chalus Branch, Islamic Azad University, Chalus, Iran

<sup>2</sup>Department of Laboratory Science, Chalous Branch, Islamic Azad University, Chalous, Iran

<sup>3</sup>Department of Microbiology, Tonekabone Branch, Islamic Azad University, Tonekabone, Iran

<sup>4</sup>Department of Internal Medicine, School of Medicine, Babol University of Medical Science, Babol, Iran

\*Corresponding author: Department of Laboratory Science, Chalous Branch, Islamic Azad University, Chalous, Iran. Email: haniyehfakhar@yahoo.com

Received 2019 June 08; Revised 2020 February 12; Accepted 2020 February 29.

## Abstract

**Objective:** Different types of *Streptococcus gallolyticus* are associated with malignant colorectal cancer. This study aimed at scrutinizing the *S. gallolyticus* screening using polymerase chain reaction (PCR).

**Methods:** A descriptive study was conducted to detect *S. gallolyticus* in 55 patients with colon diseases referring to hospitals in Babol and Chalus, Iran. PCR and culture techniques were performed. Detection of *S. gallolyticus* after DNA extraction from designed primers (PCO3 and PCO4) was done for SODA gene. Fisher's exact test was used for the analysis of PCR results. A  $P < 0.05$  was considered significant.

**Results:** Of 55 biopsy samples of patients with colon diseases, 3 samples (5.5%) with 95% confidence interval and 52 samples (94.5%) were reported negative in terms of DNA of *S. gallolyticus*. In the analysis of PCR gels, the remaining 406 bp band after performing PCR showed that all samples were in good condition considering DNA quality.

**Conclusion:** Specific probes have been designed from *S. gallolyticus* 16S rD. PCR techniques now allow for rapid identification of *S. gallolyticus* strains for clinical and other scientific investigations in colon disease.

**Keywords:** *Streptococcus gallolyticus*, Colon Diseases, PCR

## 1. Background

The human intestine, as a vital anatomical location, has the largest number of microbes; thus, it is not hard to predict the leading role of the microbes as well as the bacteria in health status and different colon diseases in this organ, especially in the development of colorectal cancers (1). Several clinical studies have demonstrated a strong association between invasive infections with *Streptococcus gallolyticus* and colon neoplasia in humans (2). Colorectal cancer is the fourth most common cancer in the world (3). The incidence of this cancer varies in different areas. In the United States and England, colorectal cancer is the second most common cancer after breast cancer in women and prostate cancer in men (4, 5). In Iran, the number of patients developing colorectal cancer is increasing, and it is prevalent in both genders (6). The colon is a hollow muscular tube that starts from the end of the small intestine or ileum and ends in the anus. The length of the intestine is 1.5 m, and its largest diameter is reported to be at the beginning of the large intestine. Its diameter decreases to the

rectum (7). The human's intestine is naturally exposed to  $10^{14}$  microorganisms, which can be hazardous to people's health and cause intestinal diseases (8). Therefore, identifying certain microorganisms enables us to improve our knowledge for the diagnosis, prevention, and treatment of these cancers (9-12). *S. gallolyticus* (*Streptococcus bovis* biotype 1) and *Helicobacter pylori* can cause colorectal cancer (13). *Streptococcus gallolyticus* subsp. *gallolyticus* (Sg), formerly known as the *S. gallolyticus*, is an opportunistic human pathogen that causes bacteremia and endocarditis. This pathogen stimulates cancer cells through the  $\beta$ -catenin signaling pathway (14, 15), and naturally lives in the human gastrointestinal tract. About 2.5% to 15% of healthy people bear this pathogen (16, 17). During the last four decades, the relationship between bacteria and colorectal cancer has been studied using serologic testing. However, the molecular findings have raised controversies. All of these studies have demonstrated that intestinal bacterial infection is associated with an increased risk of colorectal cancer and is mainly related to a bacterial agent, including

viruses, bacteria, and parasitic agents that may contribute to colorectal cancer (12). Polymerase chain reaction (PCR) is the basis for detecting *S. gallolyticus* genome in colorectal cancer, in which the tissue is detached by colonoscopy. A part of the bacterial gene is identified, and the relevant primers are produced, which confirm the final diagnosis for existing bacteria inside the colon. Moreover, culturing this Gram-positive bacterium and comparing it with the molecular method provide us with a better diagnosis (18). Recent advances in bacterial systematics have allowed for the use of the 16S ribosomal RNA (rD A) gene as a target for the detection and identification of various microorganisms. The present report describes the development of specific primers that target the 16S rD A gene to rapidly identify and differentiate human and ruminal strains of *S. gallolyticus* (19).

However, a recent study conducted in Germany using sensitive PCR to detect *S. gallolyticus* indicated a higher carriage rate estimated at 62.5% in the stools of 99 healthy volunteers (20). So far, no study has been performed to detect the colonization of *S. gallolyticus* of the large intestine directly in colorectal cancer tumors using advanced PCR molecular testing in northern Iran.

## 2. Objectives

The current study aimed at identifying the molecular structure of *S. gallolyticus* bacteria in patients with colon diseases.

## 3. Methods

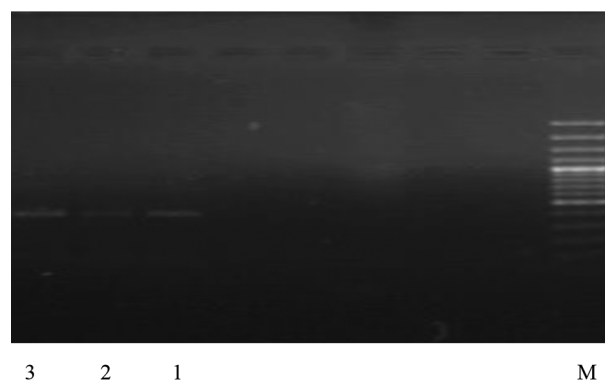
This descriptive research was conducted on 55 tissue samples of people with colon diseases undergoing colonoscopy in two hospitals of Babol and Chalous for eight months from February 2017 to September 2018. Written informed consent was obtained from patients prior to sampling. A checklist was used to collect data, including demographic information, such as age, sex, duration of illness, place of residence, family history of the disease, and having clinical symptoms, such as abdominal pain, rectal bleeding, fatigue, history of rectal hemorrhage, history of hemorrhoids, constipation, diabetes, and colonoscopy findings (polyp size, site, and histopathology). Patients with missing clinical information and incomplete colonoscopy examination were excluded. The tissue samples were collected by colonoscopy by a specialist physician for DNA extraction and performing PCR using a microtube (2 cc) containing sterile physiology serum to be sent to the molecular laboratory at -20°C.

### 3.1. Polymerase Chain Reaction

For DNA extraction, 25 mg of tissue from each sample was dried with a few drops of liquid nitrogen. DNA extraction from the chopped tissue was performed according to the instruction of the FAVORGEN kit (Taiwan) by column method using FATG2 buffers, K proteinase, and ethanol. Ultimately, to dissolve the extracted DNA, the Elution Buffer available in the kit was used. The DNA extraction was performed quantitatively ( $1.6 < OD < 1.9$ ) and qualitatively using PCO3 (5'-ACACAAGTGTGTTCACTAGC-3') and PCO4 (5'-CAACTTCATCCACGTTACC-3') primers, which duplicate a part of the human  $\beta$ -globulin gene.

The primers were developed by Primer-Blast from NCBI and Primer3 software and synthesized by the Sinoclon company, and received as lyophilized.

Using 5'-CAATGACAATCACCATGA-3' and 5'-TTGGTGCTTTTCTTTGTG-3' primers, the amplification and identification of the considered genes were performed. After preparing the final volume of each PCR reaction (20  $\mu$ L), the vials were placed in the thermocycler machine. The temperature and time condition for a PCR reaction for the genes were performed. Finally, the detection and photographing of PCR products under UV light after electrophoresis of the products for 2 to 4 minutes with agarose gel containing 1 - 0.5  $\mu$ g/mL of Ethidium bromide were performed (Figure 1).



**Figure 1.** Electrophoresis of PCR products on the agarose gel. M: a band of 100 bp and numbers 1, 2, and 3 indicating the presence of *S. gallolyticus* infection.

### 3.2. Statistical analysis

The results were analyzed by SPSS 16 software and Fisher test for the analysis of PCR results. A  $P < 0.05$  was considered significant.

#### 4. Results

In this descriptive study, 55 patients with a mean age of  $52.7 \pm 13.9$  years were included. The demographic characteristics and distribution of *S. gallolyticus* in culture samples are presented in Table 1, according to the clinical symptoms and characteristics of the patients. Table 1 signifies a significant relationship between family history of colorectal cancer ( $P = 0.011$ ) and diabetes, ( $P = 0.008$ ) and frequency of *S. gallolyticus*.

From 55 biopsy samples of patients with colon diseases, 3 samples (5.5%) were positive and 52 cases (94.5%) were negative for the presence of *S. gallolyticus* DNA. Fisher's exact test showed a significant correlation between the prevalence of *S. gallolyticus* and colon diseases ( $P = 0.015$ ). Eleven cases (20%) had a history of familial colorectal cancer, of whom 5 cases (45.5%) were observed with the bacteria ( $P = 0.011$ ). In the analysis of PCR gels, a band of 406 bp after the PCR process showed that all of the samples were in a favorable condition considering the quality of the extracted DNA. The presence of different bands in the sample indicates the presence of *S. gallolyticus* bacteria. The electrophoresis of PCR products was done on agarose gel 1.5% (Figure 1).

#### 5. Discussion

Laboratory findings of this study on PCR revealed that of 55 samples of biopsy tissues (polyps, colitis, cancer, and inflammation of the intestines), 5.5% were positive for *S. gallolyticus*. In a study by Sarokhani et al., PCR showed the presence of bacterial genomes in 36 positive samples out of 100 samples (21). A study by Gilermo on 568 patients over 65 years of age identified 15 cases with *S. gallolyticus* bacteria (22). In another study, Pui-ying et al. discovered that out of 537 patients, 12 positive samples (2.3%) were positive using PCR, and 15 cases were positive after culturing (23). Therefore, the positive predictive value for the diagnosis of *S. gallolyticus* bacteria is higher using the molecular method. Rhoads et al. found that the majority of bacteria that were recognized by the molecular method were not detectable by the culture method (24, 25). Other studies also showed that the PCR method had a higher sensitivity for bacteria diagnosis (26, 27).

In our study, 11 cases (20%) had familial colorectal cancer, of whom 5 cases (45.5%) had the bacteria ( $P = 0.011$ ). Furthermore, Norfleet and Mitchel reported a positive prevalence of *S. gallolyticus* 3% in the biopsy samples of colorectal cancer, 2.5% in normal tissue of the intestine, and 0.0% in

polyp samples (28). Using frequency-based molecular techniques, Abdulmir et al. found 48.7% of DNA sequences of *S. gallolyticus* in samples of patients with colorectal cancer in contrast to 4% of normal samples (29). Other studies have shown that *S. bovis* is associated with colorectal adenocarcinoma, especially in female patients, which contradicted our findings (30). According to the mentioned studies, the association between *S. gallolyticus* and colorectal cancer is still controversial. The differences in results can be traced to the genetic background as well as the geographical differences of the patients.

On the other hand, 20 out of 55 subjects (36.4%) had diabetes, of whom 7 subjects (35%) had *S. gallolyticus* bacteria ( $P = 0.008$ ). In a study by Zammit in 2013 on patients suffering from colon diseases, it was realized that 26% of the subjects had diabetes in addition to *S. gallolyticus* infection (31). In a case-control study, Ellezin reported a Streptococcus infection in a diabetic patient who had colon cancer (32).

##### 5.1. Conclusion

Molecular techniques have created a highly sensitive antibacterial framework bed for the detection of microbial pathogens. The sensitivity and specificity of PCR depend on the primer used. However, one of the most important factors in using PCR is the infections that can occur during DNA extraction, which is the preparation of the reaction mixture and the extension. Nonetheless, considering the advantages and disadvantages as well as the characteristics of this method, at present, this method can be considered comprehensive and standard. Thus, the simultaneous use of this method is recommended whenever a quick result is sought, or there is a suspicion of sample infection and late-growing microorganisms.

##### 5.2. Limitation and Suggestion

Due to the time limit of more than 50 cases, access to the samples of people with colon cancer and other clone diseases was not possible. We suggest further investigations to confirm the role of *S. gallolyticus* in the development of colon cancer in the future using a larger sample size (patients) compared with healthy controls.

#### Footnotes

**Authors' Contribution:** All authors equally contributed in design, statistical analysis, and manuscript writing.

**Conflict of Interests:** The authors declare no conflict of interest.

**Table 1.** Demographic Characteristics and Frequency of *Streptococcus gallolyticus* Bacteria in Culture Samples<sup>a</sup>

Specificity	Subjects	Positive Cases	Negative Cases	P Value
<b>Age, y</b>				0.999
< 50	21 (38.2)	3 (14.3)	18 (85.7)	
51 - 60	17 (30.9)	3 (17.6)	14 (82.4)	
> 60	17 (30.9)	3 (17.6)	14 (82.4)	
<b>Sex</b>				0.572
Female	26 (47.3)	4 (15.4)	22 (84.6)	
Male	29 (52.7)	5 (17.2)	24 (82.8)	
<b>Abdominal pain</b>				0.330
Yes	36 (65.5)	7 (19.4)	29 (80.6)	
No	19 (34.5)	2 (10.5)	17 (89.5)	
<b>Rectal bleeding</b>				0.577
Yes	20 (36.4)	3 (15.0)	17 (85.0)	
No	35 (63.6)	6 (17.1)	29 (82.9)	
<b>Fatigue</b>				0.373
Yes	19 (34.5)	4 (21.1)	15 (78.9)	
No	36 (65.5)	5 (13.9)	31 (86.1)	
<b>History of rectal hemorrhage</b>				0.390
Yes	8 (14.5)	2 (25.0)	6 (75.0)	
No	47 (85.5)	7 (14.9)	40 (85.1)	
<b>History of familial colorectal cancer</b>				0.011
Yes	11 (20.0)	5 (45.5)	6 (54.5)	
No	44 (80.0)	4 (9.1)	40 (90.9)	
<b>History of hemorrhoids</b>				0.214
Yes	8 (14.5)	0 (0.0)	8 (100.0)	
No	47 (80.5)	9 (19.1)	38 (80.9)	
<b>Constipation</b>				0.572
Yes	26 (47.3)	4 (15.4)	22 (84.6)	
No	29 (52.7)	5 (17.2)	24 (82.8)	
<b>Diabetes</b>				0.008
Yes	20 (36.4)	7 (35.0)	13 (65.0)	
No	35 (63.6)	2 (5.7)	33 (94.3)	
<b>Diagnosis of the disease</b>				0.186
1 month $\geq$	10 (18.2)	0 (0.0)	10 (100.0)	
1 month - 1 year	22 (40.0)	3 (13.6)	19 (86.4)	
$\leq$ 1 year	23 (41.8)	6 (26.1)	17 (73.9)	

<sup>a</sup>Values are expressed as No. (%).

**Ethical Approval:** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Also, the ethics committee of Azad University of Medical Sciences, Iran approved this study (Code no: IR.IAU.SARI.REC.1397.110).

**Funding/Support:** This study was funded by the Islamic Azad University, Chalus Branch.

**Informed Consent:** Informed consent was obtained from all participants included in the study.

## References

1. Thursby E, Juge N. Introduction to the human gut microbiota. *Biochem J*. 2017;**474**(11):1823–36. doi: [10.1042/BCJ20160510](https://doi.org/10.1042/BCJ20160510). [PubMed: [28512250](https://pubmed.ncbi.nlm.nih.gov/28512250/)]. [PubMed Central: [PMC5433529](https://pubmed.ncbi.nlm.nih.gov/PMC5433529/)].
2. Jahani-Sherafat S, Alebouyeh M, Moghim S, Ahmadi Amoli H, Ghasemian-Safaei H. Role of gut microbiota in the pathogenesis of colorectal cancer; a review article. *Gastroenterol Hepatol Bed Bench*. 2018;**11**(2):101–9. [PubMed: [29910850](https://pubmed.ncbi.nlm.nih.gov/29910850/)]. [PubMed Central: [PMC5990924](https://pubmed.ncbi.nlm.nih.gov/PMC5990924/)].
3. Triantafyllidis JK, Nasioulas G, Kosmidis PA. Colorectal cancer and inflammatory bowel disease: epidemiology, risk factors, mechanisms of carcinogenesis and prevention strategies. *Anticancer Res*. 2009;**29**(7):2727–37. [PubMed: [19596953](https://pubmed.ncbi.nlm.nih.gov/19596953/)].
4. Hewitson P, Glasziou P, Watson E, Towler B, Irwig L. Cochrane systematic review of colorectal cancer screening using the fecal occult blood test (hemoccult): an update. *Am J Gastroenterol*. 2008;**103**(6):1541–9. doi: [10.1111/j.1572-0241.2008.01875.x](https://doi.org/10.1111/j.1572-0241.2008.01875.x). [PubMed: [18479499](https://pubmed.ncbi.nlm.nih.gov/18479499/)].
5. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin*. 2005;**55**(2):74–108. doi: [10.3322/canjclin.55.2.74](https://doi.org/10.3322/canjclin.55.2.74). [PubMed: [15761078](https://pubmed.ncbi.nlm.nih.gov/15761078/)].
6. Azadeh S, Moghimi-Dehkordi B, Fatem SR, Pourhoseingholi MA, Ghiasi S, Zali MR. Colorectal cancer in Iran: an epidemiological study. *Asian Pac J Cancer Prev*. 2008;**9**(1):123–6. [PubMed: [18439090](https://pubmed.ncbi.nlm.nih.gov/18439090/)].
7. Rock J. *The Lind's operative gynecology*. 1. Philadelphia: JB Lippincott; 1997.
8. Boleij A, Tjalsma H. Gut bacteria in health and disease: a survey on the interface between intestinal microbiology and colorectal cancer. *Biol Rev Camb Philos Soc*. 2012;**87**(3):701–30. doi: [10.1111/j.1469-185X.2012.00218.x](https://doi.org/10.1111/j.1469-185X.2012.00218.x). [PubMed: [22296522](https://pubmed.ncbi.nlm.nih.gov/22296522/)].
9. Kostic AD, Chun E, Robertson L, Glickman JN, Gallini CA, Michaud M, et al. Fusobacterium nucleatum potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. *Cell Host Microbe*. 2013;**14**(2):207–15. doi: [10.1016/j.chom.2013.07.007](https://doi.org/10.1016/j.chom.2013.07.007). [PubMed: [23954159](https://pubmed.ncbi.nlm.nih.gov/23954159/)]. [PubMed Central: [PMC3772512](https://pubmed.ncbi.nlm.nih.gov/PMC3772512/)].
10. Rubinstein MR, Wang X, Liu W, Hao Y, Cai G, Han YW. Fusobacterium nucleatum promotes colorectal carcinogenesis by modulating E-cadherin/beta-catenin signaling via its FadA adhesin. *Cell Host Microbe*. 2013;**14**(2):195–206. doi: [10.1016/j.chom.2013.07.012](https://doi.org/10.1016/j.chom.2013.07.012). [PubMed: [23954158](https://pubmed.ncbi.nlm.nih.gov/23954158/)]. [PubMed Central: [PMC3770529](https://pubmed.ncbi.nlm.nih.gov/PMC3770529/)].
11. Gur C, Ibrahim Y, Isaacson B, Yamin R, Abed J, Gamliel M, et al. Binding of the Fap2 protein of Fusobacterium nucleatum to human inhibitory receptor TIGIT protects tumors from immune cell attack. *Immunity*. 2015;**42**(2):344–55. doi: [10.1016/j.immuni.2015.01.010](https://doi.org/10.1016/j.immuni.2015.01.010). [PubMed: [25680274](https://pubmed.ncbi.nlm.nih.gov/25680274/)]. [PubMed Central: [PMC4361732](https://pubmed.ncbi.nlm.nih.gov/PMC4361732/)].
12. Collins D, Hogan AM, Winter DC. Microbial and viral pathogens in colorectal cancer. *Lancet Oncol*. 2011;**12**(5):504–12. doi: [10.1016/S1470-2045\(10\)70186-8](https://doi.org/10.1016/S1470-2045(10)70186-8). [PubMed: [21067973](https://pubmed.ncbi.nlm.nih.gov/21067973/)].
13. Mantzaris GJ. Helicobacter pylori and colorectal cancer: Is there any link? *Ann Gastroenterol*. 2004;**17**(1):17–8. doi: [10.3748/wjg.v20.i21.6374](https://doi.org/10.3748/wjg.v20.i21.6374). [PubMed: [24914359](https://pubmed.ncbi.nlm.nih.gov/24914359/)]. [PubMed Central: [PMC4047323](https://pubmed.ncbi.nlm.nih.gov/PMC4047323/)].
14. Kumar R, Herold JL, Taylor J, Xu J, Xu Y. Variations among Streptococcus gallolyticus subsp. gallolyticus strains in connection with colorectal cancer. *Sci Rep*. 2018;**8**(1):1514. doi: [10.1038/s41598-018-19941-7](https://doi.org/10.1038/s41598-018-19941-7). [PubMed: [29367658](https://pubmed.ncbi.nlm.nih.gov/29367658/)]. [PubMed Central: [PMC5784120](https://pubmed.ncbi.nlm.nih.gov/PMC5784120/)].
15. Kumar R, Herold JL, Schady D, Davis J, Kopetz S, Martinez-Moczygemba M, et al. Streptococcus gallolyticus subsp. gallolyticus promotes colorectal tumor development. *PLoS Pathog*. 2017;**13**(7). e1006440. doi: [10.1371/journal.ppat.1006440](https://doi.org/10.1371/journal.ppat.1006440). [PubMed: [28704539](https://pubmed.ncbi.nlm.nih.gov/28704539/)]. [PubMed Central: [PMC5509344](https://pubmed.ncbi.nlm.nih.gov/PMC5509344/)].
16. Isenring J, Kohler J, Nakata M, Frank M, Jans C, Renault P, et al. Streptococcus gallolyticus subsp. gallolyticus endocarditis isolate interferes with coagulation and activates the contact system. *Virulence*. 2018;**9**(1):248–61. doi: [10.1080/21505594.2017.1393600](https://doi.org/10.1080/21505594.2017.1393600). [PubMed: [29072555](https://pubmed.ncbi.nlm.nih.gov/29072555/)]. [PubMed Central: [PMC5955193](https://pubmed.ncbi.nlm.nih.gov/PMC5955193/)].
17. Pasquereau-Kotula E, Martins M, Aymeric L, Dramsi S. Significance of Streptococcus gallolyticus subsp. gallolyticus Association With Colorectal Cancer. *Front Microbiol*. 2018;**9**:614. doi: [10.3389/fmicb.2018.00614](https://doi.org/10.3389/fmicb.2018.00614). [PubMed: [29666615](https://pubmed.ncbi.nlm.nih.gov/29666615/)]. [PubMed Central: [PMC5891635](https://pubmed.ncbi.nlm.nih.gov/PMC5891635/)].
18. Porgostaran R. *[Gentic Terminology]*. 2013. 424 p. Persian.
19. Chatellier S, Harel J, Zhang Y, Gottschalk M, Higgins R, Devriese LA, et al. Phylogenetic diversity of Streptococcus suis strains of various serotypes as revealed by 16S rRNA gene sequence comparison. *Int J Syst Bacteriol*. 1998;**48 Pt 2**:581–9. doi: [10.1099/00207713-48-2-581](https://doi.org/10.1099/00207713-48-2-581). [PubMed: [9731300](https://pubmed.ncbi.nlm.nih.gov/9731300/)].
20. Dumke J, Vollmer T, Akkermann O, Knabbe C, Dreier J. Case-control study: Determination of potential risk factors for the colonization of healthy volunteers with Streptococcus gallolyticus subsp. gallolyticus. *PLoS One*. 2017;**12**(5). e0176515. doi: [10.1371/journal.pone.0176515](https://doi.org/10.1371/journal.pone.0176515). [PubMed: [28459818](https://pubmed.ncbi.nlm.nih.gov/28459818/)]. [PubMed Central: [PMC5411088](https://pubmed.ncbi.nlm.nih.gov/PMC5411088/)].
21. Sarookhani M. Comparison of two diagnostic methods of culture and proliferation of 16srDNA in identification of cerebrospinal fluid bacteria in suspected patients with bacterial meningitis in Qazvin. *J Mazandaran Univ Med Sci*. 2009;**16**(69):14–21.
22. Zurita GM, Alejandra NH. Association of bacteremias of Streptococcus gallolyticus with the development of colorectal cancer in people 65 years of age. *MOJ Gerontol Geriatr*. 2018;**3**(5). doi: [10.15406/mojgg.2018.03.00144](https://doi.org/10.15406/mojgg.2018.03.00144).
23. Iroh Tam PY, Hernandez-Alvarado N, Schleiss MR, Hassan-Hanga F, Onuchukwu C, Umoru D, et al. Molecular detection of Streptococcus pneumoniae on dried blood spots from febrile Nigerian children compared to culture. *PLoS One*. 2016;**11**(3). e0152253. doi: [10.1371/journal.pone.0152253](https://doi.org/10.1371/journal.pone.0152253). [PubMed: [27007889](https://pubmed.ncbi.nlm.nih.gov/27007889/)]. [PubMed Central: [PMC4805257](https://pubmed.ncbi.nlm.nih.gov/PMC4805257/)].
24. Nafisi MR, Alipour Shadbad M, Rahimian GA, Karimi A. Comparison three methods, PCR method, culture and rapid urease test to detect Helicobacter pylori in the gastric biopsy tissue samples. *Med J Tabriz Uni Med Sciences Health Services*. 2016;**38**(5):70–5.
25. Rhoads DD, Wolcott RD, Sun Y, Dowd SE. Comparison of culture and molecular identification of bacteria in chronic wounds. *Int J Mol Sci*. 2012;**13**(3):2535–50. doi: [10.3390/ijms13032535](https://doi.org/10.3390/ijms13032535). [PubMed: [22489109](https://pubmed.ncbi.nlm.nih.gov/22489109/)]. [PubMed Central: [PMC3317672](https://pubmed.ncbi.nlm.nih.gov/PMC3317672/)].
26. Pongsachareonnon P, Honglertnapakul W, Chatsuwana T. Comparison of methods for identifying causative bacterial microorganisms in presumed acute endophthalmitis: conventional culture, blood culture, and PCR. *BMC Infect Dis*. 2017;**17**(1):165. doi: [10.1186/s12879-017-2264-5](https://doi.org/10.1186/s12879-017-2264-5). [PubMed: [28222703](https://pubmed.ncbi.nlm.nih.gov/28222703/)]. [PubMed Central: [PMC5320661](https://pubmed.ncbi.nlm.nih.gov/PMC5320661/)].

27. Mohammadi Tashakkori M, Kachuei R, Safara M, Ahmadi Z. Molecular identification of *Corynebacterium minutissimum* in skin lesions suspicious for erythrasma by the PCR method. *Pathobiol Res*. 2014;**17**(2):83–91. Persian.
28. Norfleet RG, Mitchell PD. *Streptococcus bovis* does not selectively colonize colorectal cancer and polyps. *J Clin Gastroenterol*. 1993;**17**(1):25–8. doi: [10.1097/00004836-199307000-00008](https://doi.org/10.1097/00004836-199307000-00008). [PubMed: [8409294](https://pubmed.ncbi.nlm.nih.gov/8409294/)].
29. Abdulmir AS, Hafidh RR, Bakar FA. Molecular detection, quantification, and isolation of *Streptococcus gallolyticus* bacteria colonizing colorectal tumors: inflammation-driven potential of carcinogenesis via IL-1, COX-2, and IL-8. *Mol Cancer*. 2010;**9**:249. doi: [10.1186/1476-4598-9-249](https://doi.org/10.1186/1476-4598-9-249). [PubMed: [20846456](https://pubmed.ncbi.nlm.nih.gov/20846456/)]. [PubMed Central: [PMC2946291](https://pubmed.ncbi.nlm.nih.gov/PMC2946291/)].
30. Andres-Franch M, Galiana A, Sanchez-Hellin V, Ochoa E, Hernandez-Illan E, Lopez-Garcia P, et al. *Streptococcus gallolyticus* infection in colorectal cancer and association with biological and clinical factors. *PLoS One*. 2017;**12**(3). e0174305. doi: [10.1371/journal.pone.0174305](https://doi.org/10.1371/journal.pone.0174305). [PubMed: [28355283](https://pubmed.ncbi.nlm.nih.gov/28355283/)]. [PubMed Central: [PMC5371321](https://pubmed.ncbi.nlm.nih.gov/PMC5371321/)].
31. Zammit SC, Azzopardi N, Ellul P. *Streptococcus gallolyticus* bacteraemia in hepatobiliary-pancreatic and colonic pathologies. *QJM*. 2014;**107**(5):355–61. doi: [10.1093/qjmed/hct261](https://doi.org/10.1093/qjmed/hct261). [PubMed: [24368857](https://pubmed.ncbi.nlm.nih.gov/24368857/)].
32. Elzein FE, Akhtar MY, Khairallah H, Albenmoussa A. *Streptococcus bovis* prosthetic valve endocarditis associated with silent colonic carcinoma. *BMJ Case Rep*. 2017;**2017**. doi: [10.1136/bcr-2017-219488](https://doi.org/10.1136/bcr-2017-219488). [PubMed: [28554883](https://pubmed.ncbi.nlm.nih.gov/28554883/)]. [PubMed Central: [PMC5612556](https://pubmed.ncbi.nlm.nih.gov/PMC5612556/)].