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Investigation of virulence factors and molecular analysis of bacteria isolated from pharynx and appendix of children with appendicitis, and their effects on appendicitis development

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Abstract

Acute appendicitis is an important pathology in children that requires urgent surgical intervention. Among the many factors that play a role in the pathogenesis of appendicitis, the role of microorganisms is important. In this study, we aimed to isolate bacteria from the appendicitis lumen and pharyngeal swab in children with appendicitis and to perform the molecular analysis of the most common species of bacteria isolated. The swab samples of 76 pediatric patients with suspected appendicitis were taken from the pharynx just before the surgery and from the appendix lumen during the surgery under sterile conditions and inoculated into appropriate media. Virulence gene characterization, molecular analysis, and genotype identification were performed on the most prevalent organism in both appendix and pharynx samples. Three groups were formed as negative appendectomy, complicated appendicitis, and non-complicated appendicitis. The same bacteria were isolated in both the pharynx and appendix lumen in 4 patients in the complicated group and in 2 patients in the non-complicated group. The most commonly isolated bacteria in the appendix lumen was Escherichia coli (*E. coli)*. Highly pathogenic B2 *E. coli* was found in the appendix, and commensal-growing A phylogroup *E. coli* was found in the pharynx. We isolated the same type of bacteria in both the pharynx and appendiceal lumen in a significant proportion of patients, especially those with complicated appendicitis. Although they were the same species, they were strains from different genotypes and phylogroups, except for one. Bacteria isolated from the appendix lumen had more pathogenicity and virulence. We think that the reason for the isolation of bacteria not found in normal throat flora in complicated patients may be the deterioration of general body resistance. Therefore, for patients who are found to be complicated during surgery, the early addition of antibiotics effective against highly pathogenic, resistant strains of *E. coli* may be beneficial.

Keywords: Appendicitis, appendix, pharynx, molecular analysis, Escherichia coli

Introduction

Acute appendicitis is an important pathology that is common in children and requires urgent surgical intervention [1]. Luminal occlusion is a critical event in the chain of events involved in the pathogenesis of appendicitis [2]. In the chain of events in the progress of appendicitis, it is not known exactly whether inflammation and infection develop due to lumen obstruction or whether lumen obstruction develops due to infection [3]. Although these claims are not fully proven, once appendicitis develops, symptoms, signs, and markers of systemic inflammation accompany the event[1, 4].

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There is no definite information about the role of microorganisms in the inflammation that occurs in the pathogenesis of appendicitis and their contribution to the progression of the disease [1-4].

If appendicitis was a systemic infection that first appeared in the chain of events in its development, it could be expected that organs other than the appendix would be affected by this infection. In this case, it can be expected that the same infectious agent will be isolated in other organs far from the appendix. Therefore, to determine whether bacteria play a role in the pathogenesis of appendicitis in children, we planned to isolate microorganisms from the appendix mucosa and the pharynx, an organ distant from the appendix.

The aim of the study is to determine which bacteria grow in the appendix lumen and pharyngeal swab in children with appendicitis, their molecular analysis, and to investigate the effect of this condition on the diagnosis and treatment process in the case of the same bacterial growth.

We believe that the growth of the same microorganisms in distantly located tissues, such as the appendix and pharynx, especially in patients with appendicitis, may be linked to the severity and complexity of the condition. In this case, it can ensure the treatment option to be designed in a more comprehensive manner, particularly for patients with complicated status. We are confident that this choice will be beneficial in promoting the early recovery of the patient and preventing potential complications.

Material and Methods

The study was carried out in a single-center İnönü University Turgut Ozal Medical Centre, Pediatric Surgery Clinic between July 2020 and April 2022. The study was approved by İnönü University Faculty of Medicine Clinical Research Ethics Committee in Malatya, Turkey (Decision number: 2020/85). The Helsinki Declaration was followed when conducting this study. All patients' who participated in the study had informed consent forms signed by their guardians were collected, and these records are kept in our clinic's archives.

Study Groups

The classification of the study groups was made according to the intra-operative macroscopic evaluation of all appendix tissues removed during the operation of the patients who were operated with a preliminary diagnosis of appendicitis. Histopathological examinations of all appendiceal tissues were routinely performed. However, two cases with negative appendicitis as a result of intra-operative macroscopic evaluation were diagnosed as non-complicated appendicitis in the histopathological evaluation and eventually considered as non-complicated appendicitis.

As a natural consequence, the cases formed three groups: negative appendectomy, non-complicated appendicitis, and complicated appendicitis. The negative appendectomy group consisted of the patients who were operated on due to a preoperative diagnosis of appendicitis but were intra-operatively determined to not have appendicitis.

The criteria for inclusion in the study were: i) patients with a clinical diagnosis of appendicitis; ii) patients aged between 4-17 years; iii) patients from whom a culture sample from the pharyngeal swab before surgery and from the appendix lumen during surgery could be taken; iv) patients who do not have a chronic disease such as diabetes or epilepsy; v) patients who have parental informed consent; vi) patients who underwent an open surgical appendectomy; and vii) patients who do not receive antibiotic treatment before being admitted to the hospital. Accordingly, 76 patients met the inclusion criteria, and the sample size of the study groups consisted of 11 patients with negative appendectomy, 55 patients with non-complicated appendicitis, and 10 patients with complicated appendicitis.

Our assessment approach for the basic diagnosis included radiographs of the abdomen and lungs, laboratory tests, and the Alvarado scoring system (ASS) for pediatric patients with abdominal pain [5]. All patients underwent the same physical as well as radiological tests alongside the routine laboratory tests. Once a diagnosis was made, general anesthesia was performed according to a standard protocol for all patients with appendicitis, taking into account the required preoperative fasting period.

Surgical Procedures

The same surgical and anesthesiology teams were performed all the surgeries. All patients with appendicitis underwent standard appendicitis surgery (open appendectomy) and medical treatment recommended by Dunn [1].

Preparation and histopathological evaluation of tissue sections

After a saline wash, the sections were put in a container with 10% formalin and left for 24 hours. Following this step, samples of appendix tissue were further split into 0.3 cm long pieces, processed according to routine procedures, and then embedded in paraffin blocks [6]. Then, using a microtome, sections of 3-5 μm thickness were taken from each paraffin block. The standard haematoxylin and eosin (H&E) technique was used to stain the sections. All H&E sections were evaluated by two pathologists with a light microscope (Eclipse E 200 MU Ri Nikon Corporation, Tokyo, Japan) at 40-400 magnification. Transmural neutrophil accumulation was considered as appendicitis. The absence of neutrophils in the mucous layer and muscular layer was accepted as not appendicitis.

Microbiology

In the study period, a total of 76 pharynx and appendicitis lumen culture samples were included in the study. Pharynx cultures were taken one hour before the surgery. Appendicitis lumen cultures were taken during the surgery. Pharynx culture samples taken by using a sterile swab (Inonu, Turkey) and abeslang were incubated at 37°C for 18-48 hours under aerobic conditions after sowing on 5% sheep blood agar medium. Microorganisms grown from throat culture samples were subcultured on 5% sheep blood agar

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medium and Eosin methylene blue agar medium (Bioanalyse, Türkiye) to obtain a pure culture of microorganisms.

Appendicitis lumen culture samples taken in sterile cups and sterile swab sticks were inoculated on 5% sheep blood agar, Eosin methylene blue agar, and chocolate brown agar medium (Bioanalyse, Türkiye). The appendicitis lumen culture samples were incubated at 37°C for 18-48 hours under aerobic and anaerobic conditions. For anaerobic conditions, a gaspak anaerobic system (MGC, Japan) was used. After the growth of the microorganisms on this agar medium under aerobic and anaerobic conditions, they were subcultured.

Microorganisms grown from pharynx and appendicitis lumen culture samples were identified by classical bacteriological methods such as Gram staining, the catalase test, the oxidase test, and the Vitek MS (Biomérieux, France) matrix-assisted laser desorption ionization time-of-flight mass spectrometry device.

Since *Escherichia coli* (*E. coli*) was the most common microorganism isolated in this study (in a total of 36 patients), they were examined by molecular analysis. Molecular analysis of each sample of *E. coli* growing in the complicated appendicitis group among these growing bacteria, isolated in both the throat and the appendix lumen, was performed.

Molecular Analysis

A multiplex technique developed by Clermont et al. [7] was used to assign strains into one of four phylogroups (A, B1, B2, D). Briefly, bacterial DNA was extracted using the QIAsymphony DSP Virus/Pathogen Kit and the QIAsymphony SP Automated Nucleic Acid Purification System (Qiagen, Germany), according to the instructions of the manufacturer. A quadruple Polymerase Chain Reaction (PCR) was used to search the chuA, yjaA, arpA, and TspE4.C2 genes as previously described [7]. Amplification was performed on the Gene Amp PCR System 9700 (Applied Biosystems, USA).

Virulence Genes Characterization

By using PCR, the presence of 12 virulence-related genes was determined. Genes encoding the production of adhesins/invasins (sfaDE, fimA, iha, papG-I, papG-II, papG-III, afaBCIII), outer membrane protein (ompT), toxin (hlyA), iron transport systems (iroN, iutA), and the transcriptional regulator gene (aggR) were searched, as mentioned previously[8, 9].

Genotype Identification

The arbitrarily primed polymerase chain reaction (AP-PCR) method was used to determine the clonal relationship between isolates grown in throat and appendix cultures of the same patients [10]. The cycle conditions: 94 °C for 5 min, 40 °C for 5 min, 72 °C for 1 min (2 cycles); 94 °C for 1 min, 40 °C for 1 min, 72 °C for 2 min (40 cycles). It was then imaged and recorded using the Kodak Gel Logic 200 Imaging System (Eastman Kodak Company, Rochester, NY, USA). Band profiles were analyzed using the GelCompar II software system (version 6.6; Applied Maths, Sint-Martens-Latem, Belgium).

Statistical methods

IBM SPSS Statistics for Windows, Version 26.0 (Armonk, NY: IBM Corp) was used for the analyses. The Shapiro-Wilk test was used to assess the normality of the quantitative data. If the data were normally distributed, they were summarized by mean±standard deviation, and comparisons were performed by one-way ANOVA (analysis of variance) and the Tukey post-hoc test. Otherwise, median, minimum, and maximum values were used as descriptive statistics, and as an omnibus test, the Kruskal-Wallis test and as a pairwise comparison method, the Conover method, were used for comparisons. Qualitative data were expressed by count and percentage; the exact p-value of Pearson's chi-square test was used for comparisons. In the tables, the differences between the groups with different superscript letters were found to be statistically significant. The two-sided significance level was accepted as 0.05 in all analyses.

Results

Patient Characteristics

The results of the comparisons among the groups in terms of patient characteristics are given in Table 1. There was no statistically significant difference among the groups regarding gender and age (p>0.05). The Alvarado scores of the groups and the length of hospital stay are shown in Table 2.

Table 1. Patient characteristics

Groups	Gender n (%)		Age
	Male	Female	Mean±SD
Negative appendectomy	6 (54.5)	5 (45.5)	9.36±4.06
Non-complicated appendicitis	37 (67.3)	18 (32.7)	11.49±3.37
Complicated appendicitis	5 (50)	5 (50)	9.60±5.21
p	0.47		0.12

Table 2. Analysis of the parameters of the diagnosis and treatment process of the groups

Groups	Pre-op Alvarado score	Operation time (minutes)	Length of hospital stay (days)	Length of intensive care unit stay (days)	Pre-op WBC (cell/mm3)
Negative appendectomy	7 (7-8)^{a}	40 (35-80)^{a}	2 (1-2)^{a}	0 (0-1)^{a}	12.55±2.28^{a}
Non-complicated appendicitis	8 (7-10)^{b}	60 (30-120)^{b}	2 (1-6)^{a}	0 (0-2)^{a}	15.88±3.59^{b}
Complicated appendicitis	9 (9-10)^{c}	73.5 (45-85)^{b}	2 (1-16)^{b}	1 (0-2)^{b}	19.85±3.03^{c}
p	**<0.001**	**0.001**	**<0.001**	**<0.001**	**<0.001**

In table, the differences between the groups with different superscript letters were found to be statistically significant.

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The condition of the growth of the same bacteria in the lumen of the pharynx and appendix

Anaerobic microorganisms were not found in the appendicitis lumen culture. This might be due to the transportation time and the lack of anaerobic conditions in these examples.

When the groups were analyzed in terms of the growth status of the same bacteria in the lumen of the pharynx and appendix, in 4 patients of complicated appendicitis group (40%) and in 2 patients of non-complicated appendicitis group (3.6%), the same bacteria were isolated both from the pharynx and the appendix lumen culture (Table 3).

Table 3. The condition of the growth of the same bacteria in the lumen of the pharynx and appendix

Groups	Growth of the same bacteria n (%)	
	No	Yes
Negative appendectomy	11 (100)	0 (0)[a]
Non-complicated appendicitis	53 (96.4)	2 (3.6)[a]
Complicated appendicitis	6 (60)	4 (40)[b]
p	0.002	

In table, the differences between the groups with different superscript letters were found to be statistically significant

Time from onset of the first symptoms to surgery

When the time from the onset of the first symptoms to surgery was examined amongst the groups, it was found to be significantly longer in the complicated appendicitis group than the others (Table 4).

When a comparison was performed in the complicated appendicitis group between the patients who had growth of the same bacteria in the pharynx and lumen of the appendix versus those who had not, it was found that the time was shorter in the group with growth of the same bacteria (p=0.010) (Table 4).

Table 4. Time from onset of the first symptoms to surgery

Groups	Time (hours) Median (Min.-Max.)	Time (hours) Median (Min.-Max.)	
Negative appendectomy (n=11)	18 (9-25)[a]		
Non-complicated appendicitis (n=55)	18 (9-25)[a]		
Complicated appendicitis (n=10)	204 (47-300)[b]	Same bacteria (n=4)	54.5 (47-98)
		Different bacteria (n=6)	232 (196-300)
p	<0.001		0.01

In table, the differences between the groups with different superscript letters were found to be statistically significant

Non-floral bacterial growth in the lumen of the appendix and pharynx

When the state of non-floral bacterial growth in the lumen of the appendix and pharynx were analyzed, there was no statistically significant difference between the groups (Table 5).

The most common microorganism growth in appendix lumen tissue was *E. coli* (n=37, 48%). The microorganism most often growing in pharynx samples was *Staphylococcus aureus* (n=7, 9%). The two microorganisms most frequently growing from both the pharynx and appendiceal lumen were *E. coli* (n=2, 33%) and *Klebsiella pneumoniae* (n=2, 33%).

Table 5. Distribution of non-floral bacterial growth in the lumen of the appendix and pharynx

Groups	Appendix n (%)			Pharynx n (%)		
	No	Yes	p	No	Yes	p
Negative appendectomy	11 (100)	0 (0)		9 (81.8)	2 (18.2)	
Non-complicated appendicitis	48 (87.3)	7 (12.7)	0.22	51 (92.7)	4 (7.3)	0.68
Complicated appendicitis	10 (100)	0 (0)		9 (90)	1 (10)	

Molecular Analysis

The results of the molecular analysis of *E. coli* isolated from the lumen of the appendix and pharynx culture of complicated appendicitis patients are shown in Table 6. These results show that *E. coli* isolated from the lumen of the appendix culture belongs to the B2 phylogroup, which has higher pathogenicity and higher virulence. *E. coli* isolated from the pharynx culture of the same patients belongs to the A phylogroup with lower virulence and pathogenicity, which has more commensal growing characteristics.

Genotype Identification

Although the same agents (*Klebsiella pneumoniae, Escherichia coli, and Streptococcus mitis*) were grown in the appendix and throat of three patients in the complicated appendicitis group, these microorganisms were found to have different genotypes (Table 6). Only in one patient with complicated appendicitis, Pseudomonas oleovorans of the same genotype was found to grow in both the throat and appendix (Table 6).

Additionally, it was found that the microorganisms found in two patients in the non-complicated appendicitis group, who had grown the same agents (*Klebsiella pneumoniae and Escherichia coli*) in their appendix and throat, also had different genotypes (Table 6).

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Table 6. Molecular analysis and genotypic identifier results of the same bacteria obtained from both pharynx and appendix swabs of patients

Groups	Bacteria	Genotype	Phylogroup
Complicated appendicitis - appendix	*Klebsiella pneumonia*	7	-
Complicated appendicitis - throat	*Klebsiella pneumonia*	7a	
Complicated appendicitis - appendix	*Escherichia coli*	1	B2
Complicated appendicitis - throat	*Escherichia coli*	1a	A
Non-complicated appendicitis - appendix	*Klebsiella pneumonia*	3	-
Non-complicated appendicitis - throat	*Klebsiella pneumonia*	3a	
Complicated appendicitis - appendix	*Pseudomonas oleovorans*	8	-
Complicated appendicitis - throat	*Pseudomonas oleovorans*	8	
Complicated appendicitis appendix	*Streptococcus mitis*	6	-
Complicated appendicitis - throat	*Streptococcus mitis*	5	
Non-complicated appendicitis - appendix	*Escherichia coli*	4	B2
Non-complicated appendicitis - throat	*Escherichia coli*	2	A

Discussion

Undoubtedly, one of the most important complications of appendicitis in children is perforation [1]. Once the perforation develops, both the duration of treatment and the possibility of developing other complications (intra-abdominal abscess, brid ileus, etc.) increase [1]. There are many factors that contribute to the formation of perforation [1, 3]. These are the time that passes after the onset of the first symptoms, the anatomical structure of the appendix, the severity of the inflammation that caused the development of appendicitis, and the severity of infection in the appendix tissue [1-3]. In our study, we found that in some of the patients with complicated appendicitis, the time from the onset of the first symptoms to the time of surgery (the moment when the perforation is detected) was shorter, and the same microorganisms were grown in the pharynx and appendix lumen cultures. In these patients, *E. coli* was the most common isolated microorganism. Although the same species of bacteria were isolated in the lumen of both the pharynx and the appendix, we found that they were of different genotypes and phylogroups, except for one. The strains isolated from the appendix lumen of complicated appendicitis patients are from a more pathogenic virulent phylogroup (B2 *E. coli*), suggesting that they may play a role in the pathogenesis of perforation. In addition, we determined that the bacteria isolated from the pharynx in the complicated appendicitis patient group were not normally found in the respiratory tract flora.

Wang et al. retrospectively examined 221 cases of extraintestinal infections of *E. coli*, and they reported that *E. coli* must have different properties in order to cause infection outside the intestines [11]. They evaluated four main phylogenetic groups of *E. coli* isolated from these cases, static biofilm formation, 14 genetic markers, and antimicrobial resistance data, as well as the immunological status of hosts. Also, they reported that, phylogenetically, Group B2 was most commonly isolated from the urinary tract in appendicitis cases with asymptomatic bacteremia. And, among the properties of isolated *E. coli*, the prevalence of papG III, fimH, sfa, iha, hlyA, cnf1, ompT, and usp was high. They also found that they had low antibacterial resistance against the fluoroquinolone group. In our study, *E. coli* was the most frequently isolated bacteria in the appendix lumen, and Group B2 was isolated from two patients in the complicated appendicitis group.

Hattori et al. found that aerobic bacterial growth was similar in both groups in a study of a total of 57 patients with acute appendicitis and patients who were operated on for colorectal cancer as the control group [12]. Also, they found that the number of *Bacillus species* increased as the progression of acute appendicitis increased [12]. We were not able to determine anaerobic bacteria in our study. We think that anaerobic bacteria cannot be determined due to a lack of suitable anaerobic conditions during transportation. We isolated *E. coli* in the lumen of the appendix of a total of 36 patients.

Stone, in a study in which he examined the peritoneal cavity flora of 114 appendectomy patients, reported that while in simple appendicitis there are mostly aerobic bacteria in the peritoneal cavity, if the appendix is gangrenous and perforated, there is a polymicrobial environment in which anaerobic and aerobic bacteria form a symbiosis life form [13]. In a study of microbiological examination of peritoneal fluid in pediatric patients with perforated appendicitis, Brook [14] reported that both aerobic and anaerobic bacteria grew in most of the 112 cases, and the most frequently isolated bacteria was *E. coli*. As a result of the study, Brook emphasized that there is mixed bacterial proliferation in the peritoneal cavity in pediatric patients with perforated appendicitis, that drainage is absolutely necessary for the treatment of this, and that polymicrobial antibiotic treatment is necessary.

Lari et al. conducted bacteriological studies in pediatric appendicitis patients [15]. They found that *Bacteriodes species*

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in particular were growing from swab samples taken from the peritoneal cavity and appendix. In another bacteriological study carried out in pediatric appendicitis patients, Jindal et al. found mixed flora consisting of aerobic and anaerobic bacteria in the appendix tissue of all cases [16]. *Bacteriodes fragilis and E. coli* were predominant in this flora. Türel et al. retrospectively conducted a microbiological study in a total of 209 pediatric perforated appendicitis patients [17]. An intraperitoneal culture sample was taken from patients, and 41 patients had bacterial growth (57 pathogens were isolated), with extended-spectrum beta-lactamase (ESBL)-positive *E. coli* being the most common microorganism [17].

In the prospective study conducted by Salö et al., in pediatric appendicitis patients, bacteria growing in histopathologically different appendicitis groups were examined in culture samples taken from the proximal and distal parts of the appendix mucosa [18]. According to the comparison between 19 patients and 3 controls, it is found that, especially in the perforated and phlegmanous groups, there was an increase in the amount of *Fusobacterium species* and a decrease in the amount of *Bacteriodes species*. However, this reduction was not statistically significant, and this pattern was not seen in the gangrenous appendicitis. In our study, we isolated a group of B2 *E. coli* with high pathogenicity and virulence from the appendiceal lumen of patients with complicated appendicitis. Richardsen et al.investigated the role of bacterial and viral pathogens in cases of appendicitis in children [2]. They claimed that bacterial and viral infections could trigger appendicitis in children. They stated *E. coli* as the most common bacterial pathogen and Adenovirus as the most common viral pathogen [2]. Bennion et al. performed a bacteriological examination of peritoneal fluid and appendicular tissue in patients with perforated and gangrenous appendicitis older than 12 years of age, and *Bacteriodes fragilis* and *E. coli* growth were detected in almost all cases [19]. Roberts performed a bacteriological research on the appendix tissue of 43 patients and divided them into two groups: control and acute inflamed appendicitis [20]. *Bacteriode species* were found to be the most common dominant flora, both in the control and appendicitis groups. As a result of his study, Roberts argued that the inflammation in the appendix tissue actually creates an unfavorable environment for bacterial growth, so it is unlikely that bacteria play a role in the etiopathogenesis of appendicitis.

In their study of complicated intra-abdominal infections in children, Newman et al. found that 92% of 123 patients had complicated appendicitis [21]. The most frequently isolated pathogen was *E. coli*. In our study, the most often isolated bacteria in the appendiceal lumen was *E. coli*. In both the pharyngeal and appendiceal lumen, the causative agent was *E. coli* in two of the four patients, and the causative agent was *Klebsiella species* in the other two. However, the *E. coli* bacteria isolated in the appendix from these patients were of the same species but from different strains.

Baron et al. investigated the presence of bacteria in both the appendicular lumen and peritoneal fluid of patients with acute and complicated appendicitis [22]. The study demonstrated that even when the appendix wall is intact, bacteria can spread out of the lumen. In the study of SML et al., in which the appendiceal lumen microbiota of children with complicated appendicitis was examined prospectively, it was found that the microbial composition of the appendix lumen was different in simple and complex cases of appendicitis [23]. In the bacteriological study of Tartar et al. on tissue culture in the appendix lumen of pediatric appendicitis patients, bacterial growth was observed in 75.3% of the patients [24]. The most common isolated microorganism was *E. coli*.

Nilsen et al. investigated the relationship between streptococcal pharyngitis and appendicitis [25]. In that study, it was found that the coexistence of streptococcal pharyngitis and appendicitis is actually extremely rare. In accordance with this, it is claimed that the probability of a negative appendectomy is very high in the presence of streptococcal pharyngitis in a patient with appendicitis. Lau et al. investigated the cultures of blood and appendix lumen swab in appendicitis patients, and found no correlation between these two culture outcomes [26]. The most common growing microorganism in the appendix was *E. coli*. In our study, the most common agent isolated in the appendiceal lumen was *E. coli*.

Streptococcus mitis (S. mitis) is a commensal bacterium found in the normal flora of the oropharynx, genital area, skin, and gastrointestinal tract in humans [27]. In our study, *S. mitis* was isolated from both the throat and appendix lumen of a complicated appendicitis patient. *S. mitis* has been reported to cause opportunistic infections, bacterial endocarditis, and septicemia in previous publications [27]. In our complicated case, we believe that sufficient conditions have been created for this pathogen to play a role in a systemic infection.

In our study, we think that the same type of bacteria isolated from the appendix lumen and the pharynx culture in children may contribute to the transformation of appendicitis into severe forms of appendicitis.

In addition, in our study, we used the Alvarado scoring system in the diagnosis phase. There are studies showing that the Alvarado score can be used in children [28]. Since we have been using the Alvarado scoring system for many years in our own clinic, we were more experienced in this scoring system. That's why we used the Alvarado scoring system in our study.

On the other hand, our study had some limitations. Although the number of patients was precalculated based on a power analysis, the fact that it was conducted in a larger population would have increased the power of our study. Additionally, isolation of anaerobic bacteria could not be achieved because of the unsuitable transportation conditions. And, molecular analysis was performed only on the bacteria isolated from the complicated appendicitis group and not for all bacteria.

Conclusion

As a result of our study, we isolated the same type of bacteria in both the pharynx and appendiceal lumen in a significant number of patients with complicated appendicitis in children. However, despite being the same species of bacteria, they were from different genotypes and phylogroups except for one, and those isolated from the appendix lumen had greater pathogenicity and virulence. We think that the reason for the isolation of bacteria that are not found in the normal respiratory tract flora in complicated appendicitis patients may be the deterioration of overall body resistance. We believe that isolating the microorganism in the pharynx, which should normally be found in the intestinal flora, indicates a serious condition. This could be indicative of the patient's declining general health and may potentially aid in selecting proper antibiotic therapy.

Conflict of Interests
The authors declare that there is no conflict of interest in the study.

Financial Disclosure
The authors declare that they have received no financial support for the study.

Ethical Approval
The study was approved by Inonu University Faculty of Medicine Clinical Research Ethics Committee in Malatya, Turkey (Decision number: 2020/85).

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