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BioFire blood culture identification 2 panel as detector of bacteria in peritoneal fluid from patients with acute appendicitis

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ABSTRACT

Background: Polymerase chain reaction is a method to detect bacterial DNA and is widely used because it delivers results within a few hours with the potential to guide postoperative antibiotic treatment. This study aims to determine if polymerase chain reaction can accurately detect bacteria in the peritoneal fluid compared with conventional culture from patients operated for acute appendicitis.

Methods: This prospective cohort study included patients above the age of 18 years who underwent laparoscopic surgery for acute appendicitis. Peritoneal samples were collected before the appendectomy procedure for conventional culture and polymerase chain reaction using the BioFire Blood Culture Identification 2 Panel for comparison. During surgery, the surgeon assessed the appendicitis as either complicated or noncomplicated.

Results: Samples from 102 patients were eligible for analysis. Twelve samples were polymerase chain reaction positive, and 14 samples were culture positive. The concordance of positive results when comparing these 2 methods was 71.4%. The most commonly found bacteria were *Escherichia coli* and *Bacteroides fragilis*. Of the 36 patients with complicated appendicitis, no bacteria were detected by either conventional culture or polymerase chain reaction in 21 (58%) of the patients. In patients with uncomplicated appendicitis, bacteria were demonstrated in 1 out of 66 (2%) patients.

Conclusion: This study suggests that polymerase chain reaction can be used to detect bacteria in the peritoneal fluid and has the potential to guide postoperative antibiotic treatment.

Introduction

Postoperative antibiotic treatment after surgery for acute appendicitis depends on whether the appendicitis is complicated or not. Although there is no universally accepted definition of complicated appendicitis, the distinction relies primarily upon the perioperative evaluation by the surgeon. A previous study has found that conventional culturing of perioperatively collected peritoneal fluid around the appendix did not reveal any bacterial growth in 42% of the cases assessed as complicated appendicitis.[1] As such, perioperative assessment alone may result in antibiotic overuse and unnecessary hospitalization for a significant number of patients undergoing appendectomy.

Although conventional culturing of peritoneal fluid is the primary method used to diagnose bacterial growth, the method is not without limitations. The most important one in an acute setting is the diagnostic delay. Alternative methods to detect bacteria in the peritoneal fluid are needed to aid in prompt decision-making regarding postoperative antibiotic treatment.

The polymerase chain reaction (PCR) technique can detect bacterial DNA within a few hours after sample collection. One limitation of the method is that it can only detect DNA sequences of bacteria included in the PCR panel compared to conventional culturing, which can detect most bacteria.[2,3]

A study from 2016 has shown that real-time (RT)-PCR is effective in detecting bacteria in peritoneal fluid collected from children with acute appendicitis. RT-PCR was also found to be more accurate than conventional culturing because of its ability to detect dead bacteria, anaerobic bacteria, and less viable strains.[3]

The BioFire Blood Culture Identification (BCID) is a panel that tests for 24 pathogens in blood, including gram-positive bacteria, gram-negative bacteria, and yeast.[4] Although it was designed for use on blood, BCID has proven to be useful in the detection of bacteria in various body fluids, including synovial fluid, pleural

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fluid, dialysates from peritoneal dialysis, cerebrospinal fluid, ascites, and bile fluid.[5-7] The BioFire Blood Culture Identification 2 (BCID2), a newly expanded panel, has since been introduced, which tests for 43 pathogens, including *Bacteroides fragilis* as the only anaerobic strain.[4]

The aim of this study was to compare the BCID2 panel with conventional culture to determine if PCR can accurately detect bacteria in the peritoneal fluid collected from patients undergoing acute appendectomy.

Materials and Methods

Study design and population

This study was a prospective cohort study that included patients older than 18 years who underwent a laparoscopic operation for acute appendicitis. Samples of peritoneal fluid from patients were collected in the period between June 2020 to January 2021 at the Department of Surgery, Odense University Hospital, and Svendborg Hospital, Denmark. None of the patients received antibiotics before sample collection.

Data collection

For all patients, data on sex, age, American Society of Anesthesiologists (ASA) score, per- and postoperative antibiotics, duration of hospital stay, and postoperative complications using the Clavien-Dindo classification was collected.

Preoperative blood samples were taken, and data on C-reactive protein (CRP) concentration, leukocytes, and neutrophil counts were collected. Elevated infectious markers were defined as a CRP value above 50 and/or leukocyte count above 11×10^9/L. The higher CRP cut-off was chosen to increase the likelihood of the elevation being due to a bacterial infection as opposed to inflammation.[8] Likewise, the leukocyte count cut-off is the defined threshold of leukocytosis.[9]

All data were entered into a Research Electronic Data Capture database hosted by the Open Patient Data Explorative Network.

Sample collection

After the establishment of pneumoperitoneum and visualization of the appendix, the surgeon reported whether the appendicitis was deemed complicated (defined as a visible perforation, any gangrene, periappendicular abscess, or local/diffuse peritonitis) or uncomplicated (phlegmonous inflammation). At least 5 mL of the peritoneal fluid around the appendix was aspirated in a closed system. In case there was not enough fluid for aspiration, 10 mL of isotonic NaCl was injected around the appendix before aspiration. The samples were marked as either “no NaCl fluid added” or “NaCl added.” After aspiration, perioperative antibiotics were administrated. The per-protocol standard antibiotic treatment was metronidazole and cefuroxime or metronidazole and piperacillin-tazobactam peroperatively as a single dose.

Sample preparation and analysis

The samples were sent to the Department of Microbiology at Odense University Hospital for conventional culture. Approximately half of the sample’s content was used for culturing, and the rest was stored at −80°C for later PCR analysis. PCR analyses using the BCID2 panel were performed after the end of the study period. The “no NaCl fluid added” samples were homogenized with a vortex mixer and tested undiluted. Two hundred μl were transferred to the BCID2 panel for testing. The “NaCl added” samples were also homogenized with the vortex mixer. Two mL was centrifuged at 1,730 G for 10 minutes, and 200 μl from the precipitate was transferred to the BCID2 panel for testing. The same operator prepared all tests for the BCID2 panel. The operator who performed the PCR analysis was blinded to the results of the conventional culture to avoid bias.

Statistics

All data are presented in numbers and percentages. The proportion of positive samples was compared between patient groups using the Fisher exact test. Concordance was calculated by comparing BCID2 PCR-positive samples with culture-positive samples.

Ethics

Informed consent was obtained from all participants. The study was approved by The Regional Committee on Health Research Ethics for Southern Denmark (ref. no S-20190186) and the Danish Data Protection Agency (ref. no. 20/1691).

Results

A total of 102 consecutive patients with histologic confirmed appendicitis and peritoneal fluid sampling before the appendectomy procedure were included. The patient and clinical characteristics of the group with complicated and uncomplicated appendicitis, as assessed by the surgeon, appear in Table I.

Conventional culture showed bacterial growth in 14 patients (Table II). In these patients, the PCR test was positive for 10 and negative for 2, whereas the data for PCR were missing in 2 patients. In 2 patients, no bacterial growth was detected by conventional culture, whereas the PCR was positive for *Streptococcus* spp. and *Bacteroides fragilis*, respectively. In 2 PCR negative samples *Escherichia coli* were detected by conventional culture. The types of bacteria detected by the 2 different methods appear in Table II. The concordance between the 2 methods was 71.4% when excluding the samples of unknown PCR status.

Thirteen (92.8%) of the 14 patients with a positive conventional culture had complicated appendicitis as assessed by the surgeon, whereas only 1 with uncomplicated appendicitis had a positive culture. The PCR test was positive for bacteria in 12 (92.3%) of 13 eligible samples from patients with complicated appendicitis. Of the 66 patients with uncomplicated appendicitis, only 1 had bacterial growth in conventional culture, and none had a positive PCR. However, in 7 cases with uncomplicated appendicitis, the data for PCR was missing. In 19 of the 36 patients with complicated appendicitis, no bacteria could be found by either conventional culture or PCR.

The Figure provides an overview of these results according to the surgeon’s assessment and infectious markers on admission.

Of the 36 patients with complicated appendicitis, 15 were pus samples collected from the peritoneum, of which 8 were positive by culture and/or PCR, and 19 were NaCl-injected samples, of which 5 were positive. Samples from patients assessed to have complicated appendicitis were more likely to have a positive culture and/or PCR result compared with patients with non-complicated appendicitis, $P < .001$.

In the complicated appendicitis group with elevated infectious markers, 15 (44.1%) had detectable bacteria in the samples, and 19 (55.9%) had no detectable bacteria.

The most commonly found bacteria was *E. coli*, which was present in 11 samples, closely followed by *Bacteroides fragilis* (*B. fragilis*) in 8. The mean time of response for conventional culture (from the laboratory receiving the sample until a culture-positive

Table I
Characteristics of the included patients

	Complicated appendicitis $N = 36$	Non-complicated appendicitis $N = 66$
Sex (female)	53 (51.9%)	
Age (y)		
Mean ± SD (range)	41.9 ± 17.9 (18–85)	
ASA classification		
- ASA I	14 (38.9%)	35 (53%)
- ASA II	20 (55.5%)	30 (45.5%)
- ASA III	2 (5.6%)	1 (1.5%)
Peroperative antibiotics		
- Intravenous metronidazole and cefuroxime	31 (86.1%)	61 (92.4%)
- Intravenous metronidazole and piperacillin-tazobactam	4 (11.1%)	5 (7.6%)
- None	1 (2.8%)	0 (0%)
Postoperative antibiotics		
- Intravenous cefuroxime	1 (2.8%)	0 (0%)
- Intravenous metronidazole and cefuroxime	3 (8.3%)	0 (0%)
- Tablet metronidazole and amoxicillin-clavulanic acid	32 (88.9%)	0 (0%)
- None	0 (0%)	66 (100%)
Preoperative CRP (mg/L), mean ± SD (range)	97.3 ± 76.4 (3.3–294)	45.6 ± 45.3 (0.5–252)
Preoperative leukocytes (10^9 per L), mean ± SD (range)	15.4 ± 4.2 (6.6–24)	13.4 ± 4.1 (6.2–24.8)
Preoperative neutrophils (10^9 per L), mean ± SD (range)	12.8 ± 3.9 (6.2–20.6)	10.7 ± 3.9 (3.8–20.8)
Hospitalization (h)*		
Mean ± SD (range)	19.1 ± 16.5 (3.4–68.9)	11.6 ± 10.9 (2.2–63.4)
Complications		
- Wound infections	3 (8.3%)	4 (6.1%)
- Bleeding	1 (2.8%)	1 (1.5%)
- Intraabdominal abscess	3 (8.3%)	1 (1.5%)
- Ileus	1 (2.8%)	0 (0%)
- Sepsis	5 (13.9%)	1 (1.5%)
- None	23 (63.9%)	59 (89.4%)

ASA, American Association of Anesthesiologists physical status.
* Hospitalization was defined as the number of hours from end of surgery to discharge.

result was reported to the clinician) was 77 hours ± 21.6 (range 46–101 hours).

None of the patients received antibiotics before surgery. Patients with complicated appendicitis received postoperative antibiotics with peroral metronidazole and amoxicillin-clavulanic acid for 3 days as standard. Three patients did not follow this standard. One received intravenous cefuroxime for 3 days, and 2 others received intravenous cefuroxime and metronidazole for 1 and 2 days, followed by tablet antibiotics.

Discussion

In our study, we found the concordance between conventional culture and PCR to be 71.4%. PCR was able to detect the same bacteria found in culture in most of the samples. In some samples, PCR was able to detect additional bacteria not found in the culture, such as the anaerobic bacteria *B. fragilis*. This is because *B. fragilis* is more likely to die before culturing, and its presence would, therefore, only be detectable by PCR. Furthermore, the growth of other faster-growing bacteria on the agar media could potentially hide the *B. fragilis* colonies, making identification more difficult. As concluded in the study by Tocchioni et al PCR was found to be better than the culture at finding anaerobic, resistant, or dead bacteria, which is in line with our findings.

The BCID2 panel was specifically chosen for our study because it included the most common enterobacteria, which we expected to find in peritoneal samples. A study by Cimpean et al[10] found the most frequently isolated bacteria in acute appendicitis cultures to be *E. coli*, bacteria from the *Streptococcus anginosus* group, *Bacteroides* spp., and *Klebsiella* spp. The BCID2 panel includes all of these bacteria, with the exception of *Bacteroides* spp., of which the BCID2 panel only includes *B. fragilis*. Three other *Bacteroides* spp. were cultured from our samples. Although the BCID2 panel could not detect these bacteria, it was, however, able to detect some of the other bacteria present in the same samples. In cases where bacteria not included in the BCID2 panel are present in the peritoneum, the use of the BCID2 panel alone could lead to a false negative result. This is a known limitation of the PCR method, as most panels are targeted toward identifying only the most common and clinically significant microorganisms.

Furthermore, a false positive PCR may result in an unnecessary antibiotic treatment, but this risk is relatively low, and the clinical impact of this is considered less significant. Meanwhile, a false negative test could represent a risk for the patient. Combining PCR with conventional culture could minimize these risks further. To our knowledge, there is not a panel specific for peritoneal fluid, but a panel produced specifically for peritoneal fluids might produce better results, if more intestinal bacterial species were included.

In a study by Altun et al[4] the FilmArray BCID panel was used to test samples from sterile body fluids, including peritoneal fluid, and the panel's identification rate was compared with conventional methods. When PCR was positive and detected a bacteria not found through culture, additional cultures were performed on selective agar plates to confirm its presence. The study found that PCR was able to identify the bacteria in samples with monomicrobial growth with a 100% identification rate, whereas in samples with polymicrobial growth, the identification rate was at 75%. The study found conflicting results between PCR and culture in 25% of samples. Similarly, our study found that the BCID2 panel was able to accurately identify the same bacteria in the peritoneum as conventional culture in most samples. However, the panel had trouble detecting *E. coli* in 5 samples. In 2 samples, *E. coli* was found in a monomicrobial growth, whereas in 3 samples, *E. coli* was found in a polymicrobial growth along with other bacteria. In the samples with polymicrobial growth, the panel detected the other present bacteria. Mico et al tested the FilmArray BCID panel on samples

Table II
The results of conventional culturing and PCR analysis in 102 patients operated for acute appendicitis in relation to complicated or uncomplicated appendicitis, sample type, preoperative CRP concentration, and the neutrophil leucocyte count

Sample number	Conventional culture	PCR	Surgeon's assessment of disease severity	Sample type	CRP mg/L	Leucocyte count (neutrophils) 10^9/L
21	*Escherichia coli*	Negative	Non-complicated	NaCl added	55	24.8 (17.9)
24	*Escherichia coli* *Bacteroides fragilis* *Streptococcus anginosus* group *Klebsiella pneumoniae* group	*Escherichia coli* *Bacteroides fragilis* *Streptococcus anginosus* group	Complicated	Pus	248	22.1 (19.2)
41	*Escherichia coli* *Klebsiella oxytoca* *Staphylococcus warneri*	*Escherichia coli* *Klebsiella oxytoca* *Bacteroides fragilis* *Streptococcus* spp.*	Complicated	NaCl added	130	10.4 (7.01)
46	*Escherichia coli* *Bacteroides ovatus* *Streptococcus anginosus* group	N/A†	Complicated	Pus	146	8.15 (6.51)
49	*Escherichia coli*	*Escherichia coli* *Bacteroides fragilis* *Streptococcus* spp.	Complicated	Pus	129	13.6 (10.1)
50	*Escherichia coli* *Bacteroides fragilis*	*Escherichia coli* *Bacteroides fragilis* *Streptococcus* spp.	Complicated	NaCl added	80	21.8 (17.9)
51	*Escherichia coli*	*Escherichia coli*	Complicated	Pus	140	12.4 (9.55)
53	*Pseudomonas aeruginosa* *Bacteroides salyersiae*	*Pseudomonas aeruginosa* *Streptococcus* spp.	Complicated	Pus	44	15.9 (14.3)
60	*Escherichia coli*	Negative	Complicated	Pus	151	23.5 (20.1)
62	*Escherichia coli*	N/a	Complicated	Pus	63	19.4 (17.3)
74	Negative	*Bacteroides fragilis*	Complicated	NaCl added	4.2	17.8 (15.3)
84	*Escherichia coli* *Bacteroides fragilis* *Bacteroides thetaiotaomicron*	*Escherichia coli* *Bacteroides fragilis*	Complicated	Pus	142	13.1 (9.36)
92	*Escherichia coli* *Bacteroides fragilis*	*Bacteroides fragilis*	Complicated	NaCl added	166	13.4 (11)
97	*Escherichia coli* *Pseudomonas aeruginosa* *Bacteroides thetaiotaomicron* *Bacteroides ovatus*	*Serratia marcescens* *Pseudomonas aeruginosa* *Streptococcus* spp.	Complicated	Pus	96	14.4 (12.7)
100	*Escherichia coli* *Bacteroides thetaiotaomicron*	*Bacteroides fragilis*	Complicated	NaCl added	170	9.77 (7.54)
102	Negative	*Streptococcus* spp.	Complicated	Pus	153	15.4 (12.3)

CRP, C-reactive protein; *PCR*, polymerase chain reaction.
* Streptococcus spp.: *Streptococcus agalactiae*, *Streptococcus pneumoniae* or *Streptococcus pyogenes* is reported as *Streptococcus* spp. (species) with the BCID2 panel.
† N/A: Results of PCR were missing.

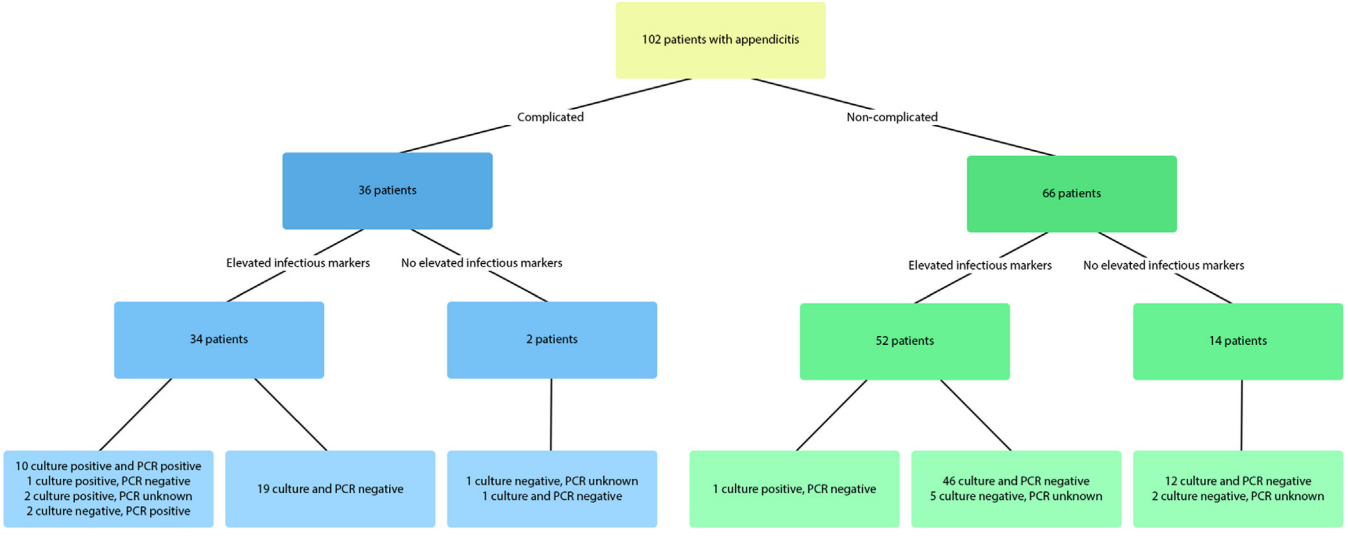

Figure. Distribution of culture and polymerase chain reaction–positive samples depending on surgeon's assessment and infectious markers.

from various body fluids and found that sensitivity was poorer in samples with low bacterial load compared with samples with high bacterial load.[7] It could be that the reason the BCID2 panel had trouble detecting *E. coli* compared to culture in some samples is the low percentage of enterobacteria in the gut compared with *Bacteroides* spp, for example.[11]

None of the patients in our study were treated with antibiotics before surgery and sample extraction. A limitation of conventional culture is that results can be affected by prior antibiotic use. Antibiotics are often administered right away in patients with acute presenting infections, ie, sepsis secondary to appendicitis, or antibiotics are administered preoperatively for prophylaxis against infectious complications, creating a gap in diagnostics that PCR would be able to bridge. Conventional culture has other limitations, such as small sample volume, slow-growing bacteria, fastidious bacteria, and inadequate storing conditions leading to false negative results,[2] by which the PCR method would be less affected. Furthermore, conventional culture involves a notable diagnostic delay. Our study found that the results of conventional culture were available to the clinician after an average of 77 hours. This diagnostic delay has the potential to affect both antibiotics use and the length of hospital stay for patients. By comparison, the BCID2 panel analyzed the samples and provided the results within an hour.

Despite the known limitations of conventional culture, it is currently our standard practice and was therefore chosen for comparison.

A total of 98.5% of the samples from patients with appendicitis assessed to be simple had no positive culture or PCR. This finding suggests that conventional culture is unnecessary in this patient group.

Meanwhile, 58.3% of the samples from patients with complicated appendicitis in our study had no positive culture or PCR. In a study by Tind et al,[1] 42% of patients assessed to have complicated appendicitis had no positive culture. In a routine surgical setting, these patients would have likely received a course of antibiotics postoperatively. Relying on PCR as a faster method to determine which patients would need antibiotics would contribute to reducing antibiotic overuse and strengthening antibiotic stewardship. The finding of a relatively low positivity rate for complicated appendicitis might call for a different clinical approach in the postoperative antibiotic treatment.

Relying on the surgeon's assessment might help narrow down who would most likely benefit from a PCR of the peritoneal fluid. We found that surgeons were overall reliable at distinguishing noncomplicated appendicitis from complicated when the outcome was the presence of bacteria in the peritoneum. The risk of a positive culture in patients assessed as uncomplicated appendicitis was very low, which makes PCR superfluous in this group of patients and without any clinical relevance. However, the surgeon's ability to distinguish seems to fall short in the complicated appendicitis group, and the use of PCR would be clinically relevant here. However, due to the possibility of false negative PCR results (as was the case with *E. coli*), we recommend combining PCR here with conventional culture.

The true diagnostic accuracy of PCR is unknown in this setting, as the aim of our study was not to evaluate the sensitivity or specificity of PCR in comparison to culture but to determine if PCR can perform as well as conventional culture in detecting bacteria in peritoneal fluid. Although we cannot make statements about the diagnostic accuracy, PCR has the potential for better guidance of postoperative antibiotics in patients with complicated appendicitis compared with usual clinical practice. We find this potential to be the most important result of our study.

Study limitations

One major limitation of this study is the small sample size of patients included. Of the 102 patients included, only 14 had positive cultures, and thus, the findings might not adequately represent the diverse population of patients undergoing appendectomy, which may affect the study's generalizability. Another limitation of the study was that the PCR status was missing for 10 patients due to insufficient or missing material for analysis. Further randomized controlled studies with a larger sample are required to properly examine the usefulness of PCR in patients with acute appendicitis, including its effect on antibiotic use, patient outcome, and health care costs.

In conclusion, this study suggests that PCR is an accurate and fast diagnostic method to detect bacteria in peritoneal fluid and is almost equal to culturing in terms of bacteria detected. A faster diagnosis of bacteria in the peritoneum could limit unnecessary postoperative antibiotics use for patients with acute appendicitis. Further, and preferably larger randomized controlled studies are needed to derive evidence-based best practices.

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Conflict of interest/Disclosure

The authors have no conflicts of interests or disclosures to report.

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