Diagnostic Microbiology and Infectious Disease 99 (2021) 115277

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Characterization of abscesses from liver, pancreas and kidney using deep sequencing of the 16S rRNA gene

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ARTICLE INFO

Article history:
Received 6 August 2020
Revised in revised form 30 October 2020
Accepted 17 November 2020
Available online 26 November 2020

Keywords:
16S deep sequencing
Next generation sequencing
16S profiling
16S rRNA
Liver
Pancreas
Kidney
Abscess

ABSTRACT

To characterize the microbial communities in abscess material from liver, pancreas, and kidneys, we performed deep sequencing of the 16S rRNA gene, in addition to cultivation and Sanger based 16S rRNA gene sequencing directly from the samples. Fifty-nine abscess samples were investigated, 38 from liver, 11 from pancreas, 10 from kidney. Using deep sequencing we made 227 bacterial identifications in 52 specimens, as compared to 69 identifications from the 44 specimens positive by culture. *Escherichia coli, Enterococcus* sp., *Klebsiella* sp. and *Streptococcus* sp. were the most common findings, but various anaerobe bacteria also constituted a large part of the microflora and those were frequently not detected by culture. Culture-independent methods like 16S deep sequencing can significantly improve microbiological diagnostics of clinical specimens. They are particularly valuable for complex purulent infections like abdominal abscesses. Therefore, deep sequencing approaches should be considered as a part of the available repertoire in diagnostic hospital laboratories.

1. Introduction

An intra-abdominal abscess is defined as a collection of pus or infected fluid, located in the peritoneal cavity and surrounded by an inflammatory wall. It may involve any intra-abdominal organ (Schein, 2001). In this study, we selected abscesses from liver, pancreas and kidney. These are often polymicrobial and represent potentially life-threatening infections with mortalities between 2.5% and 26.4% (Alvarez Pérez et al., 2001; Chan et al., 2005; Chen et al., 2005; Coelho et al., 2007; Rahimian et al., 2004).

There are many researchers studying liver abscess worldwide, with the largest number of publications coming from the United States, Taiwan, India, Japan, and South Korea (González-Alcaide et al., 2017). However, 65.9% of all documents are case reports (González-Alcaide et al., 2017). Many investigations describe treatment, symptoms and risk factors, but just a few of them go deeply into the characterization of the microbial etiology by means of metagenomic approaches (Kozlov et al., 2018; Sibley et al., 2012; Song et al., 2014). For renal and pancreatic abscesses, there is also a lack of microbiology studies and none of the existing publications to date (Coelho et al., 2007; Liu, 2016; Tsai et al., 2008) have included 16S deep sequencing data.

Conventional microbiological diagnostics are based on in vitro cultivation of specimens and identification of cultured isolates. Culture-independent approaches like amplification of the bacterial 16S rRNA gene followed by Sanger sequencing (direct 16S Sanger sequencing) are also commonly applied in routine laboratory diagnostics. Recent investigations of pleural infections, cholecystitis and brain abscesses revealed that bacterial culture only detected between 10% and 38% of the bacteria (Dyrhovden et al., 2019; Dyrhovden et al., 2020; O. Kommedal et al., 2014). Slow growing, atypical, fastidious or anaerobe bacteria like *Actinomyces* sp., *Mycoplasma* sp., *Fusobacterium* sp., *Dialister* sp., *Eikenella* sp., and many others frequently remained undetected. The same studies showed that direct 16S Sanger sequencing detected between 22% and 61% of the bacteria. This method has significant limitations in multispecies samples where resulting chromatograms can be too complex and uninterpretable (Salipante et al., 2013) and signals from minor subpopulations can be completely outcompeted in the sequencing reaction (Ø. Kommedal et al., 2011). Next-generation sequencing (NGS) of the 16S rRNA gene (16S deep sequencing) with a high number of reads per sample does not have these limitations and in principle enables identification of all bacteria present in the sample.

The aim of this investigation is to describe the bacteriology in a larger collection of liver, kidney and pancreas abscesses. In order to report thorough characterizations, we performed cultivation, direct 16S rRNA Sanger sequencing and 16S deep sequencing.

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https://doi.org/10.1016/j.diagmicrobio.2020.115277

2. Materials and methods

2.1. Ethics statement

This study was performed on remnant sample material from our routine diagnostics. The study has been approved by the Regional Committee for Medical and Health Research Ethics for the western region, Norway (REK 2017/851).

2.2. Clinical samples

We prospectively collected remnant material from liver abscesses, pancreas abscesses and kidney abscesses over a 2-year period, from April 2017 to August 2019. All relevant samples (from patients >18 years old) received for routine microbiological diagnostics in our laboratory were included. No exclusion criteria were set. Altogether, 59 samples were included, 38 samples from liver abscesses, 11 samples from pancreas abscesses, and 10 samples from kidney abscesses. Moreover, urine samples were available for all patients with renal abscess and blood culture samples were available for 39 patients (23 with liver abscess, 10 with kidney abscess, 6 with pancreas abscess). The time period between urine collection and renal pus aspiration varied from 2 days to 2 weeks. All samples were collected as part of patients' routine care and had been sent to the Department of Microbiology, Haukeland University Hospital in Bergen, Norway, for routine microbiological diagnostics. The Department of Microbiology covers a population of ~ 500 000 people.

2.3. Sample processing

Samples were investigated by culture-based routine diagnostics according to standard procedures. Abscess material was cultured on microbiological agar plates for aerobe bacteria (Columbia Blood Agar, Lactose agar with bromothymol blue, Mannitol Salt Agar), anaerobe bacteria (Fastidious Anaerobe Agar) and yeast (Sabouraud Dextrose Agar and Chromogenic Culture Media Candida (CHROMagar Candida), except for 8 samples, where cultivation of yeast had not been performed. All plates were incubated for 2 days. All bacterial isolates were identified using MALDI-TOF MS Microflex (Bruker Biotyper, Bremen, Germany). Sanger-based 16S rRNA gene sequencing was performed directly on all of the studied samples as described previously (Ø. Kommedal et al., 2011). Remnant extracted DNA was routinely stored in a −80°C biobank archive and later retrieved for metagenomics analysis.

2.4. Next generation sequencing of partial 16S rRNA gene

Next generation sequencing was performed using the Illumina MiSeq system (Illumina; Redwood City, CA). We used the Illumina protocol for 16S deep sequencing (Illumina, 2013) with the same modifications as described previously (Dyrhovden et al., 2019). The 16S rRNA primers used were forward 340F (5′-CCTACGGGNGGCWG-CAG-3′) and reverse 784R (5′-GACTACCAGGGTATCTAAKCC-3′). A negative control consisting of lysis buffer and PCR-grade (Polymerase Chain Reaction) water was extracted and analyzed in parallel with each of the samples.

2.5. Sequence data analysis

Following sequencing, FASTQ-files for each sample was analyzed individually using the RipSeq NGS software (Pathogenomix; Santa Cruz, CA). Sequencing reads shorter than 300 bp and any remnant primer sequences were trimmed away before clustering of reads into operational taxonomic units (OTUs) with a 99.0% similarity threshold. OTUs with less than 50 sequences were not considered. For taxonomic assignments, we followed the guidelines provided by CLSI (CLSI, 2018). In general, we required ≥99.0% homology with a high-quality reference combined with a minimum distance of >0.8% to the next alternative species for a valid species level identification, but the CLSI recommendation lists has a number of exceptions from this recommendation that we also took into account when appropriate. For OTUs with a homology between 95.0% and 99.0% with a high-quality reference a genus-level identification was accepted.

3. Results

3.1. Technical sequencing data

The average number of sequencing reads per sample was 427,851. After removal of short reads (<300 base pairs), small clusters (<50 reads) and chimeras, the average number of valid reads was 206,158 per sample (range 32,243−1,683,750; median 195,937).

3.2. Microbiological findings

Forty-four samples (74.6%) were positive by culture; 29 from liver, 9 from kidney, and 6 from pancreas. Forty-six samples (78%) were positive by Sanger-based 16S rRNA gene sequencing; 30 from liver, 10 from kidney, and 6 from pancreas. Using massive parallel sequencing, relevant bacterial DNA was found in 52 samples (88.1%); 34 from liver, 10 from kidney, and 8 from pancreas. In addition, we identified yeast by culture in 8 samples. Culture independent methods for yeast-detection were not included in this project. Out of 227 bacteria detected by 16S deep sequencing, culture detected 69 (31%) and Sanger-based 16S rRNA gene sequencing detected 96 (43%). The 227 microbes represented 117 different species whereof 76 were identifiable to the species level, 27 to the species group level and 14 to the genus level. Table 1 provides an overview of the most common bacteria identified in all intra-abdominal abscesses.

Four bacterial identifications were made exclusively by culture; 2 *Escherichia coli,* 1 *Enterococcus hirae*, and 1 *Staphylococcus warneri.* Eleven (28%) blood cultures were positive (6 liver patients, 4 kidney patients, 1 pancreas patient).

The Supplementary Material (Table S1) summarizes identifications made by culture, Sanger based 16S rRNA sequencing and 16S deep sequencing in all examined samples.

3.2.1. Liver abscesses

Among patients with liver abscesses, 12 samples (35%) were monomicrobial containing *E. coli* (4)*, Klebsiella* sp. (3), *Actinomyces israelii* (1), *Enterococcus* sp. (1), *Fusobacterium* sp. (1)*, Cutibacterium acnes* (1) and *Streptococcus constellatus* (1). Twenty-two samples (65%) were polymicrobial with high microbial diversities (Fig. 1). A summary over all microbes found in liver abscesses is provided in Table 2. Out of the 77 species identified, only 20 were found in more than one sample.

3.2.2. Kidney abscesses

Seven samples (70%) from kidney abscesses were monomicrobial; *E. coli* (3), *Enterobacter cloacae* complex (2) and *Klebsiella pneumoniae* (2). In 5 of these, we identified the same bacteria in urine. Three samples were polymicrobial (Fig. 1). One sample contained *E. coli* and *Staphylococcus epidermidis,* both confirmed by culture, PCR and NGS. In another sample, we cultivated only *Enterococcus faecium* whereas NGS identified 18 species whereof 14 were anaerobic or nutritionally fastidious bacteria. The last polymicrobial sample contained *Streptococcus agalactiae, Streptococcus anginosus, Prevotella bivia*, and *Veillonella atypica* (culture and NGS) and *Dialister* sp., *Enterococcus faecalis* and *Porphyromonas uenonis* (NGS only).

3.2.3. Pancreas abscesses

Four pancreas abscesses were monomicrobial with *Bacillus smithii* (1), *Citrobacter* sp. (1), *Pseudomonas aeruginosa* (1) and *Streptococcus*

Table 1
The thirteen most common genera identified in intra-abdominal abscesses.

Bacteria	Total		Liver (*n* = 38)		Pancreas (*n* = 11)		Kidney (*n* = 10)	
	n	%	n	%	n	%	n	%
Escherichia	18 (16/14)	30.5	14 (12/11)	36.8	0	0	4 (4/3)	40
Streptococcus	15 (15/7)	25.4	10 (10/5)	26.3	4 (4/1)	36.4	1 (1/1)	10
Enterococcus	12 (11/8)	20.3	9 (8/6)	23.7	1 (1/1)	9.1	2 (2/1)	20
Klebsiella	12 (11/9)	20.3	8 (7/6)	21.1	2 (2/1)	18.2	2 (2/2)	20
Prevotella	9 (9/2)	15.3	5 (4/1)	13.2	3 (3/0)	27.3	1 (1/1)	10
Staphylococcus	9 (8/7)	15.3	7 (6/6)	18.4	1 (1/0)	9.1	1 (1/1)	10
Dialister	8 (8/0)	13.6	6 (6/0)	15.8	1 (1/0)	9.1	1 (1/0)	10
Bacteroides	7 (7/1)	11.9	5 (5/1)	13.2	1 (1/0)	9.1	1 (1/0)	10
Clostridium	7 (7/1)	11.9	5 (5/1)	13.2	1 (1/0)	9.1	1 (1/0)	10
Campylobacter	6 (6/0)	10.2	4 (4/0)	10.5	2 (2/0)	18.2	0	0
Cutibacterium	6 (6/4)	10.2	6 (6/4)	15.8	0	0	0	0
Enterobacter	6 (6/3)	10.2	2 (2/1)	5.3	1 (1/0)	9.1	3 (3/2)	30
Fusobacterium	6 (6/4)	10.2	6 (6/4)	15.8	0	0	0	0

n = number of genus identifications in each category; % = the prevalence of each genus in each sample category; numbers in parenthesis represent identifications made using NGS/cultivation respectively.

constellatus (1) respectively. Four samples were polymicrobial, all of them with a diverse flora of anaerobic and nutritionally fastidious bacteria (Fig. 1). Three samples were negative by all methods.

4. Discussion

In this study, we performed microbiological characterization of 3 types of intra-abdominal abscesses supported by 16S deep sequencing. In routine laboratory practice, characterization is typically based on cultivation alone, sometimes supplemented with direct 16S Sanger sequencing.

For the monomicrobial samples, culture mostly provided a correct result (77%). However, culture was found to be insufficient in polymicrobial samples. Overall, we managed to culture only 31% of all bacteria present. Similar investigations on other types of purulent infections obtained culture success rates of 10% to 38% (Dyrhovden et al., 2019; Dyrhovden et al., 2020; O. Kommedal et al., 2014). In 4 samples however, deep sequencing failed to detect a

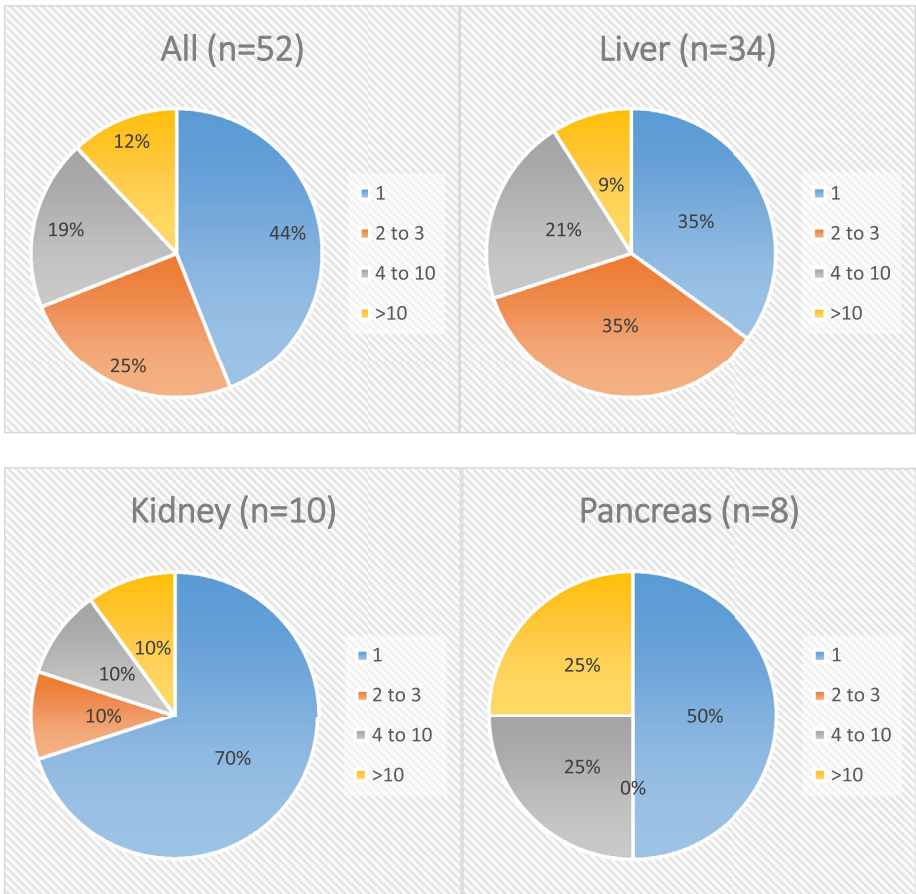

Fig. 1. Diagrams illustrate percentage of monomicrobial and polymicrobial positive samples. Polymicrobial samples are divided in 3 groups, samples containing 3 or 3 species, 4 to 10 species and more than 10 species.

Table 2
Bacteria and fungi identified in liver abscesses using 16S rRNA deep sequencing, 16S rRNA gene Sanger PCR and cultivation.

Microbes	Total number of identifications
Escherichia coli/Shigella sp.[a]	14
Klebsiella pneumoniae/variicola/quasipneumoniae[a]	7
Cutibacterium acnes	6
Fusobacterium nucleatum/naviforme/canifelium[a]	6
Staphylococcus epidermidis/capitis/caprae[a]	6
Candida albicans[b]	5
Clostridium perfringens	5
Enterococcus faecium[a,c]	5
Streptococcus salivarius group[a]	5
Bacteroides fragilis	4
Dialister invisus	4
Enterococcus faecalis	4
Campylobacter gracilis	3
Streptococcus intermedius/anginosus[a]	3
Bacteroides stercoris	2
Corynebacterium tuberculostearicum	2
Enterobacter cloacae complex	2
Granulicatella adiacens	2
Olsenella uli	2
Prevotella nigrescens	2
Acidaminococcus intestini	1
Acinetobacter calcoaceticus/pitti/lactucae/nosocomialis[a,d]	1
Actinomyces israelii	1
Actinomyces sp. (1AG30-1_×10)/*lingnae*	1
Actinomyces sp. (sp4-iso-1_H03 × 4)	1
Aggregatibacter aphrophilus/paraphrophilus	1
Anaeroglobus geminatus	1
Atopobium parvulum	1
Bacillus smithii	1
Bacteroides thetaiotaomicron/faecis	1
Bacteroides uniformis	1
Bacteroides vulgatus	1
Bifidobacterium dentium	1
Bilophila wadsworthia	1
Campylobacter rectus/showae	1
Candida glabrata[b]	1
Capnocytophaga sp.	1
Cutibacterium granulosum	1
Dialister pneumosintes	1
Dialister propionicifaciens	1
Edwardsiella hoshinae/tarda	1
Eggerthella lenta	1
Enterococcus canintestini/saigonensis[a]	1
Enterococcus hirae[b]	1
Eubacterium infirmum/Peptostreptococcaceae	1
Fretibacterium fastidiosum	1
Fretibacterium sp. (oral taxon 360)/*fastidiosum*	1
Gemella morbillorum	1
Klebsiella michiganensis/oxytoca	1
Lachnospiraceae [*G-7*] sp. (oral taxon 163)	1
Lactobacillus delbrueckii	1
Lactobacillus fermentum	1
Lactobacillus iners	1
Lactobacillus rhamnosus/casei/chiayiensis/paracasei/zeae	1
Megasphaera micronuciformis	1
Morganella morganii	1
Parvimonas micra	1
Peptostreptococcus stomatis	1
Phascolarctobacterium faecium	1
Prevotella bivia	1
Prevotella denticola	1
Prevotella oris	1
Prevotella salivae	1
Pseudomonas aeruginosa	1
Saccharomyces sp.[b]	1
Salmonella enterica	1
Selenomonas noxia	1
Shuttleworthia satelles	1
Slackia exigua	1
Staphylococcus warneri[b]	1
Stenotrophomonas maltophilia	1
Streptococcus constellatus	1
Streptococcus equinus/infantarius/lutetiensis[a]	1
Streptococcus mitis group[a]	1
Sutterella sp.	1
Veillonella parvula/dispar[a]	1
Veillonella sp. (oral taxon 780)	1

[a] Not possible to discriminate to species level using 16S rRNA sequencing
[b] Detected only by culture
[c] *Enterococcus durans/faecium/hirae/ratti/villorum*
[d] Identified by MALDI-TOF as *Acinetobacter baumannii* complex

species found by culture. This can be explained by the often undercommunicated excellent sensitivity of culture when a sample contains viable bacterial cells from a species that thrives on routine agar plates in the lab. In this situation, the sensitivity of culture can outperform that of PCR and sequencing. Although a PCR in theory requires only a single copy of the target gene to become positive, this still typically corresponds to 50 to 100 target copies per milliliter of sample material due to the low sample volume input in a PCR reaction (typically 2–5 μL) combined with the relative inefficiency of current DNA extraction methods. Another possible explanation is that although we applied a high number of reads per sample the number of reads might still have been insufficient in some of the strong positive polymicrobial samples to pick up all low abundant participants. A complete comparison for the results obtained by all methods for all samples can be found in Supplementary Table S1.

Better results can be achieved using Sanger based direct 16S rRNA gene sequencing, but this approach has severe limitations related to polymicrobial samples. Next generation sequencing has the capability to detect all present microorganisms, irrespective of their relative amounts and vitality and has previously been used to determine microbial communities in comparable studies (Dyrhovden et al., 2019; Dyrhovden et al., 2020; O. Kommedal et al., 2014; Kozlov et al., 2018; Sibley et al., 2012; Song et al., 2014). To the best of our knowledge, this is the largest collection of liver abscesses that has been characterized using next generation sequencing to date. It is also the first description of renal and pancreas abscesses using 16S deep sequencing.

In this study, the most common pathogens in liver abscesses were *E. coli* and *Streptococcus* sp. followed by *Enterococcus* sp. (Table 1). The predominance of those 3 appears to be more typical for European countries (Serraino et al., 2018). *K. pneumoniae* is the major pathogen of liver abscesses in Asia and had been reported in 55.5% to 93.4% of patient-cases (Chan et al., 2005; Song et al., 2014; Tsai et al., 2008). It has also been reported as the most frequent pathogen in the United States (41%), maybe due to a large population of Asian ethnicity (Rahimian et al., 2004). We found *K. pneumoniae* in 8 samples (21.1%) whereof 3 monomicrobial. These 4 bacteria were also reported as the most common findings in cholecystitis (Ruben Dyrhovden et al., 2020).

Fusobacterium nucleatum is known as a pathogen in brain abscesses, empyema, and periodontal infections and has recently also been reported in cholecystitis (Dyrhovden et al., 2019; Dyrhovden et al., 2020; Kommedal et al., 2014). We identified *F. nucleatum* in 6 liver abscesses. In one of these, *F. nucleatum* was the sole pathogen. According to a review of literature, this is uncommon, but *F. nucleatum* has been reported as a causative pathogen of liver abscess among immunocompromised patients with dental problems (Ahmed et al., 2015; Hammami et al., 2018; Nagpal et al., 2015). Therefore, hematogenous spread from an oral focus could represent a

plausible explanation for some liver abscesses that today are classified as cryptogenic. An autopsy case report from Japan suggests intra-arterial spreading to the liver from periodontal lesions via venous circulation, which supports this hypothesis (Ohyama et al., 2009). We also identified *F. nucleatum* in 5 polymicrobial samples. The microbial composition of these were more similar to microbial patterns reported for cholecystitis (Dyrhovden et al., 2020), and also included *Enterobacteriaceae* and *Enterococcus* making the biliary tract a more plausible origin.

Staphylococcus sp. is a common finding in liver abscesses (Serraino et al., 2018), although it has been suggested that this can represent contamination during aspiration of samples (Song et al., 2014). All of the *Staphylococcus* sp. identified in this study was coagulase-negative staphylococci. We found *Cutibacterium acnes* in four samples together with *Staphylococcus* sp. For all those cases, we had clinical information that the patient had undergone surgery and that samples therefore possibly had been collected through an indwelling catheter.

For liver abscesses, the observed variability in both complexity and composition probably reflects the many possible origins of the infection; biliary tract diseases and/or infections, hematogenous (secondary infection), portal (appendicitis, diverticulitis, sigmoiditis), complication after surgery (Lardière-Deguelte et al., 2015; Pang et al., 2011; Rahimian et al., 2004; Serraino et al., 2018), or cryptogenic. It is a limitation of this study that we did not have access to detailed clinical information including the clinically suspected origin.

Bacteria from within the family *Enterobacteriaceae* have been reported as the most common bacteria in pancreatic infections (Bjornson, 1991). Nevertheless, in this study anaerobe bacteria represented the greatest part of all identified bacteria (61.7%). Moreover, in one case we identified *Mycoplasma salivarium,* which will not grow on standard media. Polymicrobial samples constituted 50% of the positive samples. Pancreas abscesses are thought to have etiologies similar to those of liver abscesses. Pathogens can infect pancreas through the biliary tract or hematogenously (Widdison et al., 1994). It has also been reported that microbes can spread from the transverse colon (Widdison et al., 1994). Therefore, it is not unexpected that most of the identified microorganisms are known as part of a normal gastrointestinal microbiota.

Renal abscesses are often a complication of urinary tract infections. The correspondence between bacteriological findings in urine and in abscesses is up to 93.4% (Coelho et al., 2007). The most common pathogens found in urine from patients with renal infection are *Escherichia coli* and *K pneumoniae* (Coelho et al., 2007), but *Staphylococcus aureus* is also quite often reported, especially among children (Liu, 2016). We found a clear association between renal abscesses and urinary tract infection in 5 cases. In the remaining 2 samples, we identified a great number of bacteria including anaerobes. Therefore, we hypothesize a different origin of infection for these latter 2 cases.

In addition to the lack of clinical information, it is a limitation of the study that the number of patients was low for pancreas and kidney abscesses, and investigations were performed just in one research unit. Therefore, our results need verification by other studies with larger number of included patients.

5. Conclusions

Many bacterial species normally assumed to be of clinical relevance were frequently not detected by cultivation. Therefore, culture independent methods may be considered as a supportive diagnostics tool in laboratory practice in some cases. Moreover, rare and uncommon bacteria should not be forgotten by clinicians, especially in immunocompromised patients. Knowledge about the microbial communities in different types of infections is important to assure good empiric treatment recommendations.

Author statement

Joanna Malgorzata Bivand designed the study together with Øyvind Kommedal, performed the data-analysis and wrote the first version of the manuscript.

Randi Monsen Nygaard was responsible for the 16S deep sequencing experiments.

Øyvind Kommedal contributed in the creation of the manuscript and supervised the project.

Declaration of competing interest

All authors declare no conflicts of interest.

Acknowledgments

This work was supported by the Department of Microbiology, Haukeland University Hospital, Norway. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.diagmicrobio.2020.115277.

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