



A search strategy for detecting duodenoscope-associated infections: a retrospective observational study

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SUMMARY

Background: Duodenoscope-associated infections (DAIs) are exogenous infections resulting from the use of contaminated duodenoscopes. Though numerous outbreaks of DAI have involved multidrug-resistant micro-organisms (MDROs), outbreaks involving non-MDROs are also likely to occur. Detection challenges arise as these infections often resolve before culture or because causative strains are not retained for comparison with duodenoscope strains.

Aim: To identify and analyse DAIs spanning a seven-year period in a tertiary care medical centre.

Methods: This was a retrospective observational study. Duodenoscope cultures positive for gastrointestinal flora between March 2015 and September 2022 were paired with duodenoscope usage data to identify patients exposed to contaminated duodenoscopes. Analysis encompassed patients treated after a positive duodenoscope culture and those treated within the interval from a negative to a positive culture. Patient identification numbers were cross-referenced with a clinical culture database to identify patients developing infections with matching micro-organisms within one year of their procedure. A 'pair' was established upon a species-level match between duodenoscope and patient cultures. Pairs were further analysed via antibiogram comparison, and by whole-genome sequencing (WGS) to determine genetic relatedness.

Findings: Sixty-eight pairs were identified; of these, 21 exhibited matching antibiograms which underwent WGS, uncovering two genetically closely related pairs categorized as DAIs. Infection onset occurred up to two months post procedure. Both causative agents were non-MDROs.

Conclusion: This study provides crucial insights into DAIs caused by non-MDROs and it highlights the challenge of DAI recognition in daily practice. Importantly, the delayed manifestation of the described DAIs suggests a current underestimation of DAI risk.

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Introduction

Endoscopic retrograde cholangiopancreatography (ERCP) is a minimally invasive procedure for treating bile duct, liver, and pancreas diseases. ERCPs are performed with duodenoscopes, which are side-viewing endoscopes with a complex design. Duodenoscopes undergo high-level disinfection to eliminate micro-organisms; nevertheless, studies show that 5–15% of ERCPs are performed with contaminated duodenoscopes [1,2]. The incidence of post-ERCP sepsis ranges from 0.3% to 5.4%, with a mortality rate of up to 29.4% [3]. These infections can be endogenous or exogenous in origin. Endogenous infections arise from translocation of the patient's own flora during the procedure. There is an increased risk of translocation of micro-organisms, as ERCPs nowadays have evolved into more invasive and therapeutic interventions [4]. When micro-organisms, not originating from the patient, are introduced via the environment or a contaminated duodenoscope, an exogenous infection may occur. If the duodenoscope is the source of the causative bacterium of the infection, this is referred to as a duodenoscope-associated infection (DAI), which is, by definition, a healthcare-associated infection.

Over the last two decades, DAIs have been increasingly reported, primarily in the context of outbreaks and involving multidrug-resistant organisms (MDROs) [5]. A recent study revealed a DAI base risk estimation of 0.01%, markedly surpassing prior assumptions [6,7]. However, the actual risk of DAI is likely even higher [5,6]. Factors contributing to the under-recognition of DAIs include infrequent duodenoscope surveillance, potential time lags between the procedure and the onset of infection, or a clinical picture with only mild symptoms [3,5]. Moreover, not all detected outbreaks are consistently reported [8]. DAIs that involve susceptible micro-organisms often go unrecognized, contributing to the underestimation of DAI incidence. Currently, only a few studies have been published on this topic, and the clinical impact of susceptible micro-organism transmission through contaminated duodenoscopes remains poorly understood [9–11].

Microbiological surveillance of duodenoscopes is a recommendation in most guidelines [12–14]. If duodenoscopes are found to be contaminated with gastrointestinal flora, they are quarantined to prevent patient exposure. However, contamination may have occurred at any point between two culture moments. Assuming that cultures accurately represent the duodenoscope's microbiological status, any patient undergoing an ERCP between negative and positive culture results could potentially have been exposed to a contaminated duodenoscope, putting them at risk of DAI. The objective of this study was to identify patients who developed a DAI within one year following their ERCP procedure.

Methods

Setting

The Erasmus MC is a large tertiary care centre, conducting around 750 ERCP procedures on adult patients per year. We analysed the culture results of duodenoscopes between March 2015 and September 2022, encompassing three distinct duodenoscope models: the Olympus TJF-160VR, the Pentax ED34-i10T, and the Pentax ED34-i10T2 equipped with disposable

endcaps. This study was approved by the Erasmus MC medical ethical committee (MEC-2022-0767).

Data collection

Duodenoscope culture data was obtained from the laboratory system of the Department of Medical Microbiology and Infectious Diseases (MMID). The dataset included the duodenoscope identification number, sample collection date, duodenoscope sample site, cultured micro-organisms, and colony-forming units (cfu). A duodenoscope culture was defined as the combined result of the sample sites of the duodenoscope. The maximum number of sample sites varied depending on the duodenoscope type, ranging from five to six. These sites included the suction channel, biopsy channel, brush passed through the biopsy and suction channel, air/water channel, forceps elevator (area)/distal tip, and, if applicable, the forceps elevator channel and protective cap. Duodenoscope usage data was gathered from the Endobase system (Olympus, Hoofddorp, Netherlands) or electronic patient records.

Reprocessing and microbiological surveillance

The methods employed for reprocessing duodenoscopes, sampling duodenoscopes, and culturing have been published [15].

Search strategy

All duodenoscope cultures that tested positive for gastrointestinal flora were identified, regardless of cfu count. These data were paired with the duodenoscope usage database to pinpoint patients who had been exposed to contaminated duodenoscopes. Precise determination of when a duodenoscope became contaminated was not possible since detection depended on the culturing moment. Therefore, the study included not only all patients treated after a positive duodenoscope culture but also those treated within the culture interval leading up to the positive culture with gastrointestinal flora.

After identifying (potentially) exposed patients, their patient identification numbers were linked with a database containing all clinical cultures processed by the MMID. This enabled the identification of patients who developed infections with micro-organisms of the same species as those found in the duodenoscope culture within one year of their ERCP. We set a one-year cut-off because there are no studies describing the development of duodenoscope-associated infections (DAIs) beyond that period and to manage the data volume for analysis. The culture database encompassed all registered patient materials, including blood, urine, bile, sputum, and ascites. A 'pair' was defined as a match on species level between the duodenoscope culture and the patient culture.

Pair analysis

All pairs were identified and the presence of micro-organisms in isolate storage collections was checked for. If the isolate of interest was cultured from multiple duodenoscope sample sites within the same duodenoscope culture set, the micro-organisms found in the sample sites were prioritized

in the following order: forceps elevator/distal tip, biopsy channel, suction channel, brush passed through the channels, and lastly the air/water channel. Additionally, for each infection episode, only one isolate from the patient cultures was chosen for analysis.

We compared the micro-organisms within the pairs based on their antibiograms. If the antibiogram was not yet available in the laboratory information system of the MMID, we retrieved the micro-organisms of interest from storage. Subsequently, these isolates were plated on blood agar (BD, Drachten, Netherlands), incubated overnight at 35 °C, and the antibiogram was obtained using Vitek2 (bioMérieux, Marcy l'Etoile, France). Two medical microbiologists were involved in determining whether the micro-organisms matched based on the antibiotic susceptibility patterns. Pairs that matched on antibiogram underwent whole-genome sequencing (WGS) using Illumina technology generating >100× coverage (Novogene, Cambridge, UK). Genomic assemblies were created in CLC Genomics Workbench v22 using default parameters (Qiagen, Hilden, Germany). Subsequently, core-genome multi-locus sequence typing (cgMLST) was performed to assess genetic relatedness using the species-specific schemes available in SeqSphere+ Software (Ridom, Munster, Germany). For *Enterobacter ludwigii* and *Enterobacter hormaechei*, no cgMLST scheme was available and therefore an ad-hoc cgMLST scheme was created for both species using the genome sequence of their strains as seed genome and other genomic sequences available from the National Centre of Biotechnology Information as penetrating genomes. *Enterobacter* spp. isolates were identified to the species level by analysing their genomic assembly using the Type Strains Genome Server (tygs.dsmz.de) [16]. If the isolates within a pair displayed close genetic relatedness, defined as having fewer than 20 allelic differences or being identical, they were classified as either definitive or probable cases of DAI. A definitive DAI was determined when the duodenoscope culture tested positive before the patient culture. Conversely, a probable DAI occurred when the patient culture yielded a positive result before the duodenoscope culture.

Statistical analyses

Categorical variables are presented as absolute or relative frequencies. No advanced statistical analysis was performed.

Results

A total of 1803 patients were identified as (potentially) exposed to contaminated duodenoscopes. Among them, 68 (3.8%) patients had developed an infection within one year after their ERCP, caused by a micro-organism matching at species level with the culture of the duodenoscope that was used for their treatment. Thus, 68 pairs with isolates from duodenoscope and patient culture were created and were deemed suitable for further analysis. Thirty-one (45.6%) pairs could not be further analysed because at least one of the isolates of interest was not stored. From the remaining 37 pairs, isolates from 21 (30.9%) pairs also matched based on antibiogram and underwent WGS. Following cgMLST analysis, one definitive DAI and one probable DAI were identified (Figure 1).

Case 1

On March 29th, 2017, a Pentax ED34-i10T duodenoscope (Duodenoscope 1) tested positive with *Enterobacter cloacae* complex. Subsequently, the duodenoscope was placed under quarantine until June 2nd, 2017. During the quarantine period, the duodenoscope underwent three cultures on April 26th, May 28th, and June 1st. The culture conducted on April 26th revealed the presence of the *E. cloacae* complex and *Enterococcus* sp., whereas the last two cultures, conducted on May 28th and June 1st, showed no gastrointestinal micro-organisms. Following the lifting of the quarantine, two additional cultures were collected on July 3rd and July 18th. The culture obtained on July 18th showed again a positive result for *E. cloacae* complex.

On June 14th, 2017, Patient 1 underwent an ERCP for obstruction complaints by suspected Bismuth IV cholangiocarcinoma. A long stenosis extended from the left hepatic duct to the common hepatic duct up to the cystic duct level. The stenosis was treated by dilation and stent placement in the left biliary system, but no clinical improvement occurred. Another ERCP was performed on July 20th, using Duodenoscope 1, to replace the previous stent with a larger one. On July 25th, the patient underwent a portal vein embolization of liver segment four. The remaining post-procedure recovery was uneventful and adequate biliary drainage was achieved.

On September 4th, 2017, the patient underwent a right hemihepatectomy with double hepaticojejunostomy and Roux-Y anastomosis. On September 9th, the patient became feverish along with redness and leakage at the cranial section of the surgical wound. Immediate intervention involved reopening the wound to allow for better drainage. Subsequent computed tomography conducted on September 12th revealed an abdominal fluid collection. Antibiotic therapy with cefuroxime and metronidazole was initiated. On September 15th, two months after the first ERCP procedure, the patient developed fever (temperature 38.8 °C) and blood cultures identified *Enterococcus faecium* and *E. cloacae* complex.

The isolates from Duodenoscope 1 on March 29th and July 18th, as well as the isolate from the patient's blood culture on September 15th, were retrieved from storage. Unfortunately, the *Enterococcus* sp. identified on the duodenoscope on April 26th was unavailable for analysis. Antibiograms indicated that the *E. cloacae* complex isolates were non-MDRO with highly similar profiles (Supplementary Table S1). Subsequently, these isolates underwent WGS. The analysis revealed that the isolates from the duodenoscope culture on March 29th and the patient's blood culture on September 15th were *E. ludwigii*. The isolate from the duodenoscope culture on July 18th, the most recent before the patient's treatment on July 20th, was identified as *E. hormaechei*. The *E. ludwigii* isolates from the duodenoscope culture and the patient culture were genetically related, differing by only seven allelic variations (Figure 2). Duodenoscope 1 had been cleaned and disinfected 35 times between March 29th and July 20th.

Patient pathology results of the resection specimen showed no signs of malignancy, only chronic inflammation. Blood tests and histomorphological and immunohistochemical analysis did not provide sufficient evidence for an IgG4-mediated disease. One year later, the patient made a full recovery and resumed full-time employment.

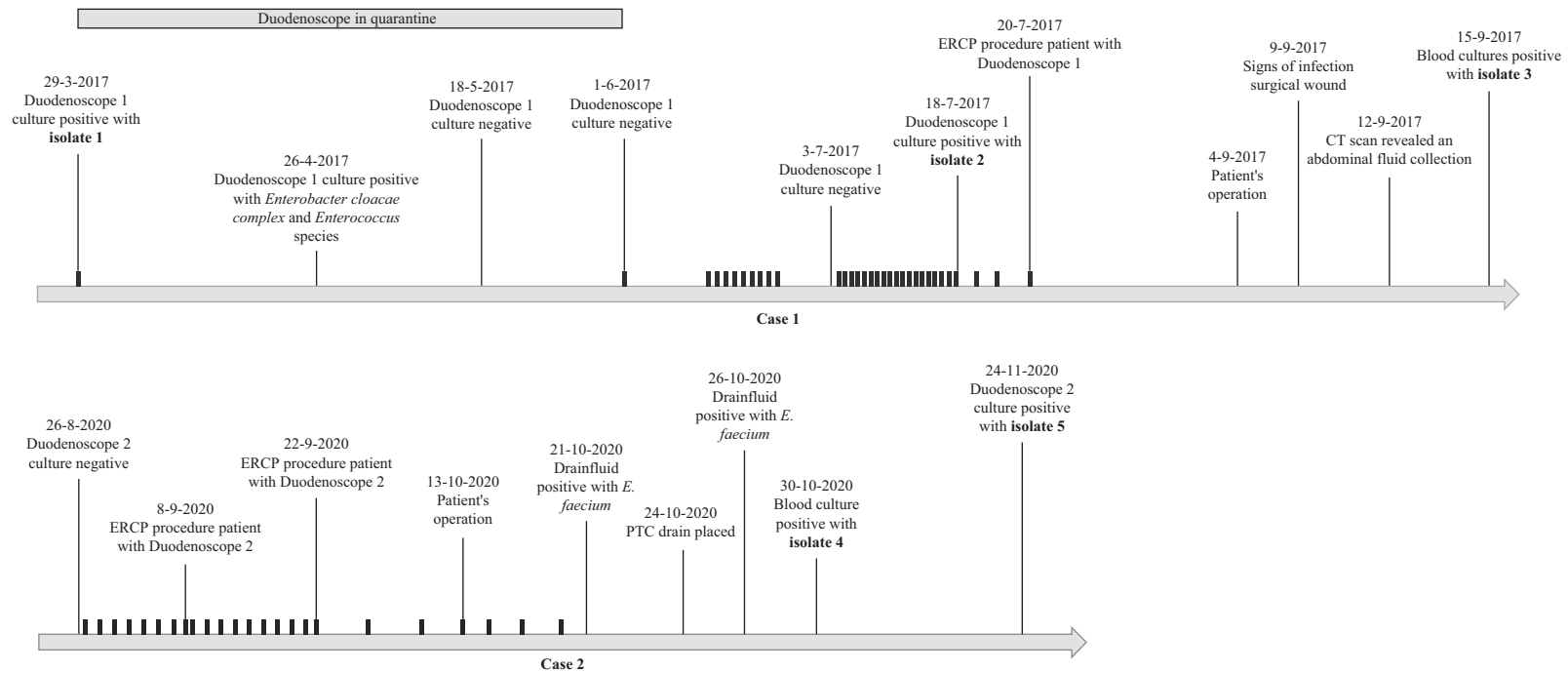


Figure 1. Timeline of definite (Case 1) and probable (Case 2) duodenoscope-associated infections. Black rectangles indicate duodenoscope use and subsequent high-level disinfection. ERCP, endoscopic retrograde cholangiopancreatography; PTC, percutaneous trans-hepatic cholangiography.

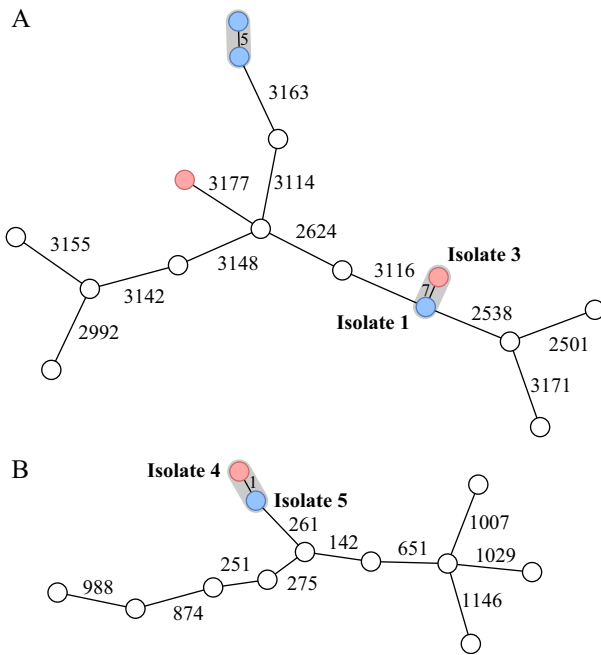


Figure 2. Minimum spanning tree illustrating genetic relatedness between isolates based on cgMLST analysis. Each circle represents a genotype. Numbers on connecting lines indicate the number of allelic differences between the genotypes. Related genotypes are indicated with a grey background. Blue genotypes indicate duodenoscope isolates. Red genotypes indicate clinical isolates. White genotypes are randomly selected genomes from the National Centre for Biotechnology Information to illustrate the overall diversity between isolates. (A) *Enterococcus ludwigii*, ad-hoc cgMLST scheme consisting of 3455 loci. (B) *E. faecium*, cgMLST published scheme consisting of 1423 loci [23].

Case 2

November 24th, 2020, a culture of the Pentax ED34-i10T2 duodenoscope (Duodenoscope 2) revealed the presence of *E. faecium*. The previous culture, obtained on August 26th, 2020, showed no contamination with gastrointestinal micro-organisms. During the period between the two sampling time points, a total of 23 procedures in 19 different patients were performed using Duodenoscope 2.

Patient 2 underwent two treatments with Duodenoscope 2 on September 8th and 22nd, 2020. Earlier, on September 4th, the patient had an ERCP for suspected biliary obstruction (with a different duodenoscope) and was diagnosed with Bismuth II cholangiocarcinoma. During this ERCP, a plastic stent was placed in the right biliary system. The ERCP on September 8th was performed to treat a bout of cholangitis by dilating the stenosis and placing plastic stents in both biliary systems. The procedure on September 22nd, prompted by ongoing cholangitis, repeated stenosis dilation and stent replacement, after which the patient recovered quickly.

On October 13th, 2020, the patient underwent a left hemihepatectomy with bile duct resection and Roux-Y reconstruction. After the operation, the patient had persistent heightened infection and liver parameters. On October 24th, 2020, a percutaneous biliary drain was inserted due to suspected hepaticojejunostomosis leakage. Six days later, *E. faecium* was isolated from blood cultures.

The antibiotic susceptibility patterns of the isolates obtained from the patient's blood cultures and from Duodenoscope 2 on November 24th were found to be identical (Supplementary Table S1). Following this observation, WGS was performed and the isolates were genetically related, differing in only one out of 1423 allelic targets. The timeline is shown in Figure 1.

Patient 2 was initially discharged in late 2020 but had two readmissions within two months due to worsening condition. In February 2021, computed tomography showed disease progression. Care was shifted to focus on palliation and the patient was discharged to a hospice facility, dying one month later.

Discussion

We present one definitive and one probable DAI case caused by non-multidrug-resistant bacteria, which were confirmed with WGS to be genetically related to those bacteria that were cultured from two duodenoscopes. This confirms a causal link, and transmission due to the ERCP procedure must have occurred. Without our comprehensive search strategy and systematic approach, the micro-organisms cultured from the duodenoscopes would never have been identified as the cause of the patients' infections. Thus, with this study we provide evidence for the existence and clinical impact of DAIs due to susceptible micro-organisms and the challenges to detect these in routine clinical practice.

Several factors make it extremely difficult to link a patient's infection to an ERCP procedure that was carried out with a contaminated duodenoscope. A significant time gap may exist between the duodenoscope's positive culture and the ERCP procedure with concurrent transmission, and subsequently development of an infection. In Case 1, false-negative cultures cleared the duodenoscope for clinical use, which resulted in a four-month interval between the duodenoscope culture and the patient's ERCP. The patient developed an infection in September, indicating a six-month gap from the duodenoscope culture and a two-month gap between the ERCP and DAI onset. In Case 2, the ERCP preceded positive duodenoscope culture. The patient developed the infection a month after the ERCP, with a two-month gap between the ERCP and duodenoscope culture and a one-month gap between patient infection and duodenoscope culture. In a previous case report of a DAI published by our research team, an infection developed three months after the ERCP [10].

Nearly all published outbreaks exclusively concern DAIs caused by MDROs [5]. This is because MDROs, unlike susceptible micro-organisms, can be recognized relatively easily due to a clustering of cases. Robust infection control measures, including active surveillance and outbreak monitoring, are often implemented to control MDRO transmission. Healthcare practitioners also maintain heightened vigilance when dealing with MDRO-related infections. Therefore, it is crucial to acknowledge the likely underestimation of DAIs caused by susceptible micro-organisms.

It is important to mention that Duodenoscope 2 tested positive for *E. faecium* after the patient's ERCP procedures. Though it is theoretically possible that this organism originated from the patient's own flora, independently contaminating the duodenoscope and later causing the patient's infection, the likelihood of this scenario is significantly lower compared to the

alternative that the isolate was already present in the duodenoscope and was introduced during the procedure. This interpretation is supported by the fact that Duodenoscope 2 had been used on seven other patients before Patient 2 following the duodenoscope culture on August 26th, 2020. Furthermore, as demonstrated in Case 1, duodenoscope cultures can yield false-negative results, leaving open the possibility that *E. faecium* was already present in the duodenoscope culture of August 26th.

Currently, it is not possible to reliably predict whether a duodenoscope will become contaminated following a procedure. Factors that are believed to contribute to the risk of endoscope contamination are delays in the initiation of cleaning, errors in the cleaning process, incomplete drying, and endoscope damage [17–19]. However, these factors are not consistently monitored, and their effect on duodenoscope contamination in the daily operations of an endoscopy department remains unclear. Also, it is unknown whether the characteristics of the ERCP procedure, such as duration or therapeutics performed, or the patient's microbiome, influence the risk of duodenoscope contamination. Despite comprehensive microbiological surveillance, it remains inevitable that patients will be exposed to contaminated duodenoscopes between culture intervals.

Additionally, the reliability of the duodenoscope cultures themselves remains uncertain. In Case 1, despite the duodenoscope testing negative on three occasions during the interim, the micro-organism found in the culture in March must have still been present at the time of the patient's ERCP procedure in July. A comparable scenario was previously documented in a case report, where six culture sets over a two-month period failed to detect the micro-organism ultimately causing a DAI [10]. In the Erasmus MC, no neutralizing agent is used during duodenoscope sampling, which may influence culture reliability [20]. Additionally, it has been suggested that it is likely that micro-organisms persist in duodenoscopes in a viable but non-culturable state, embedded within biofilms [21]. This could hinder the detection of these micro-organisms in cultures performed during the quarantine period, causing false-negative culture results. These micro-organisms, however, may release and become clinically relevant once the duodenoscope is reused. Instruments used during an ERCP, which are advanced through the working channel, are likely to disrupt the biofilm, thereby exposing the embedded micro-organisms shedding into the lumen of the working channel. Based on this assumption, there might be a need for repeated duodenoscope cultures shortly after the quarantine is lifted to ensure that cultures are truly negative.

This retrospective detection of DAI cases serves as a valuable proof of principle. However, considering the factors described above, it is crucial to recognize that our findings do not provide data on the overall incidence of DAIs. This is further strengthened by the fact that, despite our meticulous efforts, it is very likely that DAIs have unintentionally been overlooked in this study. The storage of all gastrointestinal bacteria identified in duodenoscopes is essential and adherence to storage protocols should be included in audits. Furthermore, in clinical cultures, only isolates from blood cultures are often routinely stored, while specimens from other sources such as bile or urine are not. A comprehensive assessment of the true incidence of DAIs would require a standardized follow-up protocol and active surveillance of patients that were treated with

contaminated duodenoscopes. However, such an undertaking is labour-intensive and expensive.

Despite extensive efforts, a feasible solution to mitigate the risk of DAIs has not been found yet. The development of sterile single-use endoscopes is promising; however, widespread implementation is hindered by significant cost implications and a substantial increase in environmental impact [22]. Another approach to reduce the risk of DAIs in high-risk procedures is pre-procedure duodenoscope culture. Once the duodenoscope is confirmed culture-negative, it can be reserved for high-risk patients, such as the immunocompromised. By implementing this strategy, healthcare providers can proactively minimize the chances of contamination and subsequent infection in vulnerable patients.

This study has some limitations. First, unavailable micro-organisms in storage hindered the analysis of 31 pairs. Second, analysing only one isolate per infection episode from patient cultures may have resulted in missed cases. Third, despite our acceptance of minor differences in antibiotic susceptibility during antibiogram comparisons, spontaneous isolate mutations can significantly alter antibiograms, potentially leading us to exclude them from WGS analysis.

In conclusion, this study, employing a comprehensive search and test strategy, offers evidence that clinically relevant DAIs, including those caused by susceptible micro-organisms, are challenging to identify but do occur. A substantial time gap between exposure to a contaminated duodenoscope and the onset of clinical infection may exist. There are significant knowledge gaps regarding the frequency, accurate and timely identification, and prevention of DAIs. Implementing the current search strategy in other centres could validate our findings and offer valuable insights into DAI prevalence.

Conflict of interest statement

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jhin.2024.02.015>.

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