# Bile microbiota in Pancreatic and Extra-Pancreatic Biliary Tract Cancer: A New STROBE retrospective cohort study

*Autori:* Paola Di Carlo[1*], Nicola Serra[2], Teresa Maria Fasciana[1], Francesco D'Arpa[3], Erika Di Marco [1], Consolato M. Sergi [4] and Antonio Cascio [1]

Affiliazioni:1. Department of Health Promotion, Maternal-Childhood, Internal Medicine of Excellence "G. D'Alessandro", University of Palermo, 90127, Italy 2. Department of Public Health, University Federico II of Naples, Italy. 3. Department of General Surgery and Emergency, University of Palermo, Italy. 4. Lab. Med. and Pathology, Children's Hospital of Eastern Ontario (CHEO), University of Ottawa, Ottawa, ON, Canada.

Background:
Recently, a microbial community to cancers of the pancreas and the biliary tract has been under the lens to identify a distinct microbiome composition associated with pancreatic cancer (PC) and extra-pancreatic biliary cancer (EPC).

Methods:
We investigated bile microbiota in patients with malignant pathologies of the biliary and pancreatic tract and evaluated if there is a different microbiological pattern in subjects with pancreatic and extra-pancreatic biliary tract cancers. One hundred forty-five positive bile cultures of Italian patients who underwent cholangiopancreatography (ERCP) with PC and EPC cancer hospitalized from January 2006 to December 2020 in a QA-certified academic surgical unit were investigated for aerobic, anaerobic, and fungal organisms.

Results:
In Table 1 we reported the characteristics of the 145 patients with pancreatic and extra-pancreatic cancer. Gram negative bacteria were isolated in 80% of bile cultures, both Gram positive and Gram-negative bacteria were detected in 10% of cancer patients. 12/145 (17.4%) patients showed candida spp in bile samples. In Table 2, we report the isolates individuated in this study. Particularly, some patients showed more isolates (Gram negative, Gram positive, or both). Specifically, Table 2 shows about Gram negative bacteria a significant less frequent of bacteria such as M. morganii, P. agglomerans, E. meningoseptica, Serratia spp, Delftia acidovorans, and Brevundimonas spp; while a significant most frequent were Klebsiella spp, Escherichia coli, and Pseudomonas spp. For Gram positive bacteria a significant less frequent were Staphilococcus spp and Streptococcus spp; while Enterococcus spp was the bacteria most frequent.

We observed that the PC group had a significant presence of patients of greater age than the EPC group (p=0.0351). Among Gram-negative bacteria, Escherichia coli and Pseudomonas spp were the most frequent in the EPC group. In PC group, Escherichia coli, Klebsiella spp and Pseudomonas spp were the most frequent. In addition, both groups had Enterococcus spp as the most frequent bacteria. In comparing EPC and PC groups, we observed a significant presence of Gram-negative (p=0.0005) and Candida spp in PC compared with EPC group (p=0.0032). In contrast, no significant differences for Gram-positive were found (p=0.26). In Table 3, in EPC group, among Gram negative we observed a significant less frequent of Acinetobacter spp; while E. coli and Pseudomonas spp were the Gram negative most frequent. In PC group the Gram negative significant less frequent were M. morganii, P. agglomerans, E. meningoseptica, Serratia spp, Brevundimonas spp, Alcaligenes faecalis and Enterobacter spp; while E. coli, Klebsiella spp and Pseudomonas spp were the Gram-negative bacteria most frequent. In addition, about Gram positive both groups had Enterococcus spp the most frequent bacteria. About the comparison between EPC and PC group, we observed a significant presence of Gram negative and Candida spp in PC in comparison with EPC group (53.10% vs. 23.66%, p=0.0005; 7.59% vs. 0.69%, p=0.0032, respectively), while no significant differences for Gam positive were found (p=0.26). In addition, from bivariate analysis between cancer groups and Gram negative a significant association was found (p=0.0187). In particular by post hoc z-test, it resulted that in EPC group Alcaligenes faecalis was the bacteria most frequent(p=0.039) and M. morganii was the bacteria less frequent(p=0.014), while in PC group, we found a significant less frequent bacteria such as M. morganii (p=0.0196), P. agglomerans (p=0.0196), Serratia spp (p=0.0104), Alcaligenes faecalis (p=0.002), and Enterobacter spp (p=0.0159).

Conclusions: In our pancreatic and extra-pancreatic biliary tract cancer patients, we found a more prevalent gut species of Enterobacteriaceae, well-known in gut dysbiosis. The geographical difference in gut microbiota composition may influence the biliary habitats in our cancer population. The prevalence of Candida spp in subjects with pancreatic cancer supports the hypothesis of a correlation between mycobiome, bile microbiota, and pancreatic cancer. Distinct microbiome signatures may be associated with cancer in the pancreatic and biliary habitats.

**Keywords:** Pancreatic cancer, extra-pancreatic biliary cancer, bile microbiota

Table 1. Characteristics of 145 patients with pancreatic and extra-pancreatic biliary cancer

| Parameters | Sample |
|---|---|
| *Patients* | 145 |
| *Age at microbiological analysis* | |
| Mean±SD | 75.1±10 |
| Median (IQR) | 76(69;82.25) |
| *Gender* | |
| Male | 54.5% (79) |
| Female | 45.5% (66) |
| *Cancer* | |
| Pancreatic | 64.8% (94) |
| Extra-pancreatic | 35.2% (51) |
| *Bacteria (# patients)* | 141 |
| *Gram-* | 82.8%(120) |
| *Gram+* | 3.45%(5) |
| *Both* | 11.03%(16) |
| *Fungus (# patients)* | 12 |
| *Candida spp* | 2.76%(4) |
| *Candida spp and Gram-* | 2.76%(4) |
| *Candida spp and Gram+* | 2.76%(4) |
| *Candida spp and Gram- and Gram+* | 0.0%(0) |

Table 2. Strains isolated from 145 patients with pancreatic and extra-pancreatic cancer

| Isolates on 145 patients | %(Nr) |
|---|---|
| *Gram- ( patients)* | 82.76% (120)† |
| M. morganii | 0.83%(1)*** |
| P. agglomerans | 0.83%(1)*** |
| E. meningoseptica | 1.67%(2)*** |
| Serratia spp | 1.67%(2)*** |
| D. acidovorans | 2.50%(3)*** |
| B. spp | 2.50%(3)*** |
| A. faecalis | 3.33%(4) |
| Enterobacter spp | 4.17%(5) |
| Achromobacter spp | 5.83%(7) |
| Citrobacter spp | 5.83%(7) |
| Acinetobacter spp | 6.67%(8) |
| Stenotrophomonas spp | 8.33%(10) |
| Klebsiella spp | 15.0%(18)** |
| E. coli | 24.17%(29)** |
| Pseudomonas spp | 31.67%(38)** |
| *Gram+ (patients)* | 14.48%(21)† |
| Staphilococcus spp | 4.76%(1)*** |
| Streptococcus spp | 9.52%(2)*** |
| Enterococcus spp | 90.48%(19)** |
| *Candida spp* | 8.28%(12) |

Table 3. Strains isolated from EPC and PC group

| Isolates on 145 pz. | EPC Group %(N) | PC Group %(N) | EPC vs. PC p-value (Test) |
|---|---|---|---|
| *Gram- (patients)* | 29.66% (43) | 53.10% (77) | **0.0005*(C)** |
| M. morganii | 2.33% (1) | 0.0% (0)*** | |
| P. agglomerans | 2.33% (1) | 0.0% (0)*** | |
| E. meningoseptica | 2.33% (1) | 1.30% (1)*** | |
| Serratia spp | 4.65% (2) | 0.0% (0)*** | |
| D. acidovorans | 2.33% (1) | 2.60% (2) | **0.0187* (C)** |
| Brevundimonas spp | 4.65% (2) | 1.30% (1)*** | A. faecalis (EPC)**, p=0.039(Z) |
| A. faecalis | 9.30% (4) | 0.0% (0)*** | Acinetobacter spp (EPC)***, p=0.0... |
| Enterobacter spp | 9.30% (4) | 1.30% (1)*** | M. morganii (PC)***, p=0.0196(Z) |
| Acinetobacter spp | 0.0% (0)*** | 10.39% (8) | P. agglomerans (PC) ***, p=0.019... |
| Achromobacter spp | 6.98% (3) | 5.19% (4) | Serratia spp (PC) ***, p=0.0104(Z... |
| Citrobacter spp | 4.65% (2) | 6.49% (5) | A. faecalis (PC)***, p=0.002(Z) |
| Stenotrophomonas spp | 4.65% (2) | 10.39% (8) | Enterobacter spp (PC) ***, p=0.01... |
| Klebsiella spp | 11.63% (5) | 16.88% (13) ** | |
| E. coli | 27.91% (12)** | 22.08% (17)** | |
| Pseudomonas spp | 25.58% (11)** | 35.06% (27)** | |
| *Gram+ (patients)* | 5.52% (8) | 8.97% (13) | 0.26 (C) |
| Staphilococcus spps | 0.0%(0) | 7.69% (1) | |
| Streptococcus spp | 0.0%(0) | 15.38% (2) | 0.37(C) |
| Enterococcus spp | 100%(8)** | 84.62% (11)** | |
| *Fungus (patients)* | 0.69%(1) | 7.59% (11) | |
| | | | **0.0032*(C)** |
| Candida spp | 100%(1) | 100%(11) | |

† = there are patients with more isolates;
\* = significant test;
\*\*=significant most frequent;
\*\*\*=significant less frequent.
T= t-test;
MW=Mann Whitney test was used in the case of the distribution was not normal.
C = chi-square test;
F= Fisher's exact test;
Z= post hoc z-test;

Reference: Aykut B, Pushalkar S, Chen R, Li Q, Abengozar R, Kim JI, et al. The fungal mycobiome promotes pancreatic oncogenesis via activation of MBL. Nature. 2019;574(7777):264-7. DOI 10.1038/s41586-019-1608-2
Matsukawa H, Iida N, Kitamura K, Terashima T, Seishima J, Makino I, Kannon T, Hosomichi K, Yamashita T, Sakai Y, Honda M, Yamashita T, Mizukoshi E, Kaneko S. Dysbiotic gut microbiota in pancreatic cancer patients form correlation networks with the oral microbiota and prognostic factors. Am J Cancer Res. 2021 Jun 15;11(6):3163-3175. PMID: 34249452; PMCID: PMC8263681. Sergi C, Di Carlo P, Gulotta G, D'Arpa F. Biliary microbiota in pancreatic cancer. HPB (Oxford). 2019;21(12):1790. DOI 10.1016/j.hpb.2019.06.001