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Bacterial analysis of the bile in the patients with acute cholecystitis using next-generation sequencers

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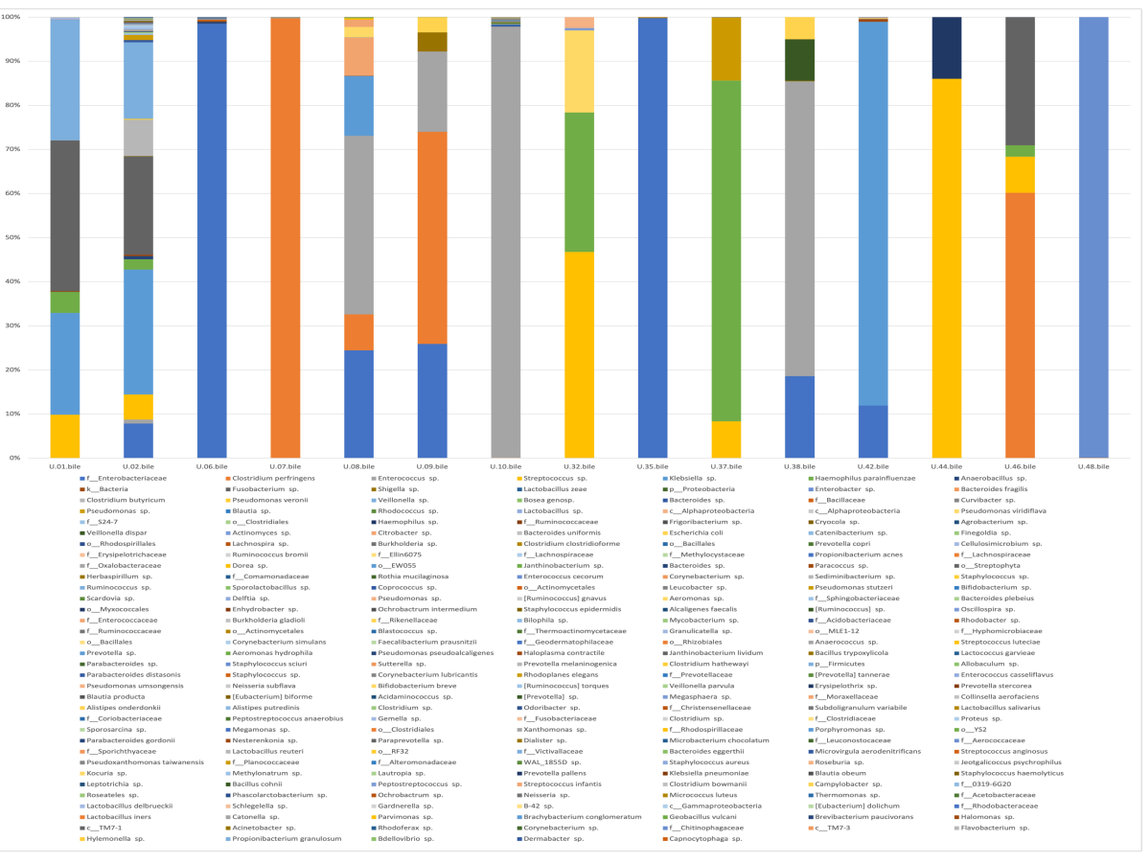
<INTRODUCTION>

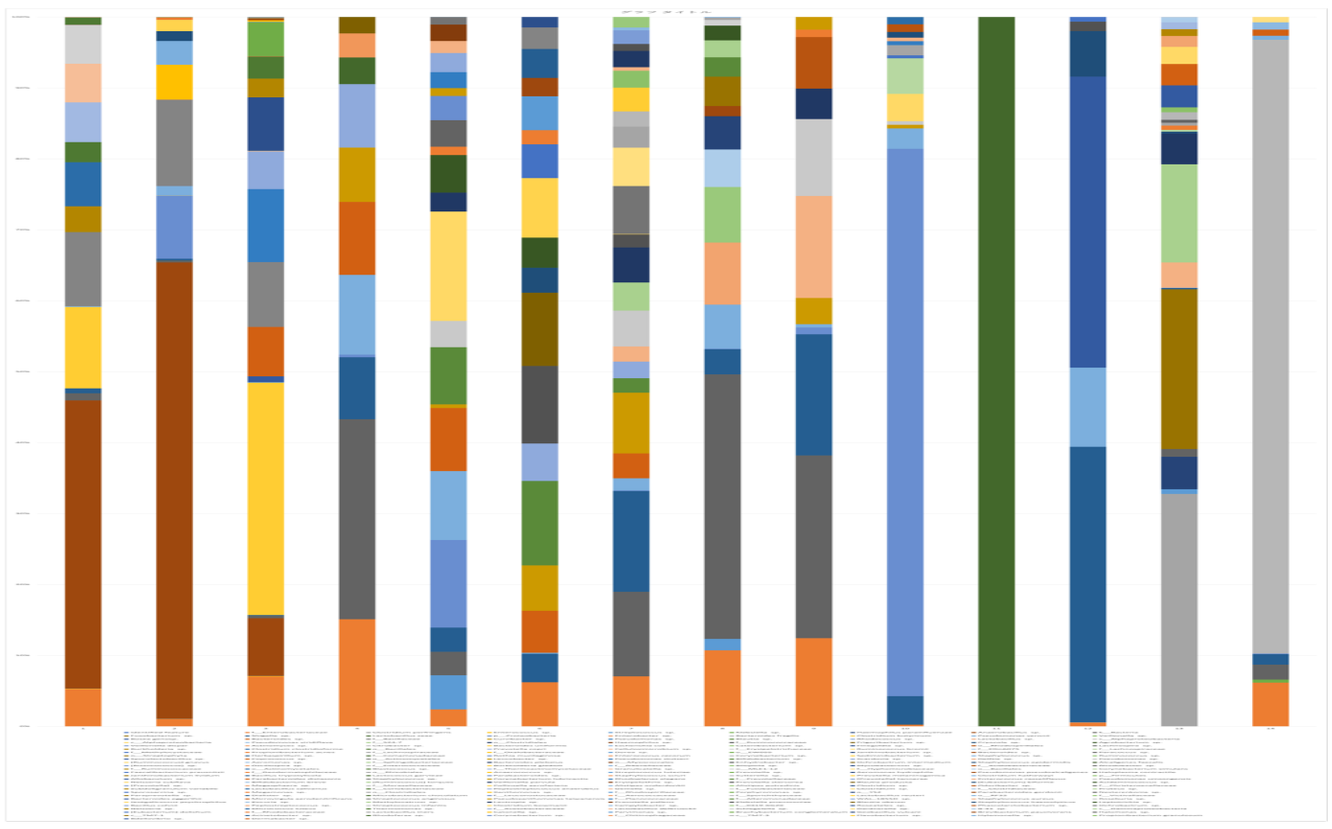
Acute cholecystitis is a common biliary tract infection. The cause of acute cholecystitis is impaction of a gallstone in the outlet of gallbladder, either cystic duct or Hartmann's pouch. The acute inflammation of cholecystitis is complicated by secondary biliary infection. However, in some patients no bacteria are detected in the usual bacterial culture. In the present study, we examined the bacterial flora of the patients with acute cholecystitis using conventional culture and 16srRAN.

<MATERIAL and METHOD>

Bile samples were collected from the gallbladders of 29 patients (twenty men and nine women; age range, 31-84 years) who underwent cholecystectomy for acute cholecystitis between July 2021 and January 2023 at Juntendo University Urayasu Hospital. Bile was frozen in an ultra-low temperature freezer as soon as possible after collection. All patients received CMZ 1g or CTM 1g as a preoperative antimicrobial prophylaxis about 30 minutes before cholecystectomy.
Bacterial DNA was extracted from bile samples using the DNeasy PowerSoil Kit (QIAGEN, Venlo, Netherlands). Specimens were stored under identical conditions and processed by trained staff using identical protocols. Samples were collected using aseptic techniques for quality control, and polymerase chain reaction (PCR) reagents were regularly checked for environmental contaminants; PCR reactions had appropriate controls (no template) to exclude DNA contaminants. To control for sequencing quality, "negative" (reagent only) controls were used to check for background contamination and incidence of sequencing errors.



Positive Bile flora(genus)



Negative Bile flora(genus)

Fourteen out of 29 patients were culture-negative by traditional culture technique. However, metagenomic analysis clearly showed the bacterial flora such as Anaerobacillus sp、Fusobacterium sp、Curvibacter sp、Corynebacterium sp、etc. were detected even in the bile that was aseptic by traditional culture.

<CONCLUSION>

In a bacterial flora analysis targeting the 16S ribosomal gene, a specific bacterial flora was detected in bile collected from the patients with acute cholecystitis even in the patients whose bile was sterilized by traditional culture techniques.