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Bactibilia in women affected with diseases of the biliary tract and pancreas. A STROBE guidelines-adherent cross-sectional study in Southern Italy

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Abstract

Purpose. Bile is a hepatobiliary lipid-rich sterile solution, and its colonization by microorganisms defines the condition of bactibilia. In this study, we aimed to assess the bile microbiological flora and its potential link with comorbidity in women.

Methodology. We performed a microbiologic investigation on 53 female patients with biliopancreatic diseases who granted consent, and we analysed the data using a MATLAB platform.

Results. We found that the most frequent disease associated with bactibilia was pancreas head carcinoma (PHC) (P=0.0015), while the least frequent disease was gall bladder carcinoma (GBC) (P=0.0002). The most common microorganisms were *Pseudomonas* spp. (P<0.0001) and *Escherichia coli* (P<0.0001). In particular *Pseudomonas* spp. and *E. coli* were negatively correlated to PHC presence and positively correlated to CCA by both univariate and multivariate analysis.

Conclusions. Gram-negative bacteria have been linked to a tumour-associated inflammatory status. In the last 30 years, the analysis of mortality rate in Italy for PHC and GBC shows an increasing and a decreasing trend, respectively. Although this study targeted only 53 patients and does not reflect the frequency of diagnosis in a Southern Italian population, the decrease in GBC may raise the suggestion ofnon-adherence to a Mediterranean diet that may have become more prevalent in Southern Italy since the 1990s.

INTRODUCTION

Women's health has improved considerably, and life expectancy will probably increase in the next half of the 21st century despite incresed microbiological and toxicological threats [1–4]. Recent reports have indicated that human commensal gut microflora may be in jeopardy owing to increased environmental pollution [5–13]. Such statements have addressed the decreased immunological response in both experimental animals and humans [13]. Gut microbiota may be particularly important because an imbalance may also affect the absorption of vitamins and alter the female skeletal composition, with an increase in osteomalacia and predisposition to cancer [14]. The relationship between human disease and biodiversity is, however, complicated, and biodiversity changes may not necessarily compromise human illness in all cases [15]. Thus, study of the relationship between the micro-environment of the digestive system and environmental factors is now more crucial than ever [11]. Moreover, bacterial strains isolated in animal food samples may substantiate the contribution of the food chain and autophagy mechanisms in the transmission of pathogens to humans [16–19]. We previously found that mucosal barrier depletion and loss of bacterial diversity are primary abnormalities in inflammatory bowel disease (IBD) [20–22]. Biliopancreatic disease and bactibilia, i.e. the colonization of bile by microorganisms, have not been studied explicitly in regard to women. New therapeutic strategies in line with more recent discoveries may be essential in guiding health care policies [23–25]. Some subgroups of female patients may harbour a higher risk of life-threatening complications such as intra-abdominal infection (IAI), and Gram-negative bacteria have been linked to an inflammatory state with a predisposition to sepsis [2, 4, 26, 27].

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Abbreviations: CCA, Cholangiocellular Carcinoma; GBC, Gallbladder Carcinoma; IAI, Intra-abdominal Infection; IBD, Inflammatory Bowel Disease; MATLAB, Matrix Laboratory; PHC, Pancreas Head Carcinoma; STROBE, Strengthening the Reporting of Observational Studies in Epidemiology.

In this study, we evaluatedbactibilia in women undergoing surgery for biliary and pancreatic diseases.

METHODS

We performed a STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines-adherent cross-sectional study on bile samples from 53 women admitted to the Paolo Giaccone University Hospital in Palermo, Italy, with follow-up data spanning a 5-years period (January 2011–December 2016). We defined study inclusion criteria as all consecutive female patients with IAIs identified according to current clinical and pathologic strategies [4] and who granted consent to their participation in the study. Bile samples were sent to the microbiology laboratory of the Department of Sciences for Health Promotion and Mother–Child Care, where species identification was determined by a BD Phoenix system [28]. Demographic data, predisposing factors and clinical, endoscopic and radiological features were collected from the electronic medical files. Microbiological records were systematically checked for matching culture reports. Any comments made at multidisciplinary team meetings were recorded in the minutes and approved. The study protocol conformed to the Declaration of Helsinki ethical guidelines for clinical studies and was approved by the local University Ethics Committee (University of Palermo, Institutional Review Board), and is adherent to the STROBE statement and guidelines [29].

The statistical analysis was performed using MATLAB toolbox version 2008 (MathWorks, Natick, MA, USA) for Windows. We used multiple comparison chi-square tests to define significant differences in a group of diseases and bacterial types. When multiple chi-square tests were positive (P-value <0.05), residual analysis and Z-test were performed to identify bacterial and disease levels. In addition, univariate and multivariate linear correlation analyses were performed. Testing for Pearson's linear correlation coefficient R was performed using Student's t-test, under the null hypothesis of Pearson's linear correlation coefficient with R equal to zero. For this step, we considered the variables *survival patients*, *disease*, *bacteria* and *age*. With regard to the *disease* and *bacteria,* we determined scores according to frequency, labelling cholangiocellular carcinoma (CCA) as 1, gall bladder carcinoma (GBC) as 2 and pancreas head carcinoma (PHC) as 3; and for bacteria: *Brevundimonas* spp.=1, *Elizabethkingia* spp.=2, *Enterobacter* spp.=3, *Enterococcus* spp.=4, *Acinetobacter* spp.=5, *Alcaligenes* spp.=6, *BGNI*=7, *Citrobacter* spp.=8, *Delphia* spp.=9, *Achromobacter* spp.=10, *Stenotrophomonas* spp.=11, *Klebsiella* spp.=12, *Pseudomonas* spp.=13 and *E. coli*=14.

Finally, the experimental probability distribution for PHC, CCA, GBC and all bacteria identified in this study was defined assigning 1 for presence and 0 for absence.

We considered significant all statistical tests with a P-value <0.05. Some early preliminary data of this study have previously been published [30].

RESULTS

Fifty-three women with diseases of the biliary tract and/or pancreas were investigated. The age range was 49–93 years [mean: 73.4 years; standard deviation (SD): 10.5 years]. Table 1 shows the demographic characteristics of our patients regarding age, survival, disease and microorganisms identified. We found that the most frequent disease was PHC (P=0.0015), while the least frequent was GBC (P=0.0002), with significant P-values using a multi-comparison chi-square test. With regard to bacteria, the most common strains were *Pseudomonas* spp. (P<0.0001) and *E. coli* (P<0.0001), with significant P-values using a multi-comparison chi-square test and residual analysis. Investigation of the correlations among age, bacteria, disease and survival time in our patients is presented in Table 2. Univariate analysis of disease type showed a positive correlation of disease with the age of the patients. Both univariate and multivariate analysis showed a negative linear correlation between disease type and survival time, with patients with PHC showing poorer survival than patients harbouring a malignant bio-pancreatic disease. Finally, we investigated possible correlations between disease type and the most frequently identified bacteria – *E. coli*, *Klebsiella* spp. and *Pseudomonas* spp. by univariate and multivariate linear correlation analysis. As shown in Table 3, we observed that *E. coli* and

Table 1. Demographic data, nosology and microorganism profile of female patients with diseases of the biliary tract/pancreas

Parameter	Mean±SD/Percentage	
Age	73.4±10.5 years	
Survival time	8.7±3.4 years	
Disease		
CCA	20/53	37.7 %
GBC	2/53	3.8 %
PHC	31/53	58.5 %
Bacterial species		
Achromobacter spp.	3/53	5.7 %
Acinetobacter spp.	2/53	3.8 %
Alcaligenes spp.	2/53	3.8 %
Brevundimonas spp.	1/53	1.9 %
Citrobacter spp.	2/53	3.8 %
Delphia spp.	2/53	3.8 %
Elizabethkingia spp.	1/53	1.9 %
Enterobacter spp.	1/53	1.9 %
Enterococcus spp.	1/53	1.9 %
E. coli	13/53	24.5 %
Klebsiella spp.	6/53	11.3 %
Pseudomonas spp.	12/53	22.6 %
Stenotrophomonas spp.	3/53	5.7 %
GNBNI	2/53	3.8 %
Fungus		
Candida spp.	2/53	3.7 %

CCA, cholangiocellular carcinoma; GBC, gall bladder carcinoma; PHC, pancreatic head cancer; GNBNI, Gram-negative bacilli not identified.

Table 2. Univariate and multivariate linear correlation analysis

Parameter	Univariate analysis	Multivariate analysis
	***R* (*P*-value)**	**Multiple linear correlation coefficient=0.869**
Disease/Age	0.278 (0.0435)*	*R* partial=0.159; *P*-value=0.265
Disease/Bacterial species	−0.042 (0.762)	*R* partial=0.088; *P*-value=0.537
Disease/Survival time	−0.865 (<0.0001)*	*R* partial=−0.857; *P*-value<0.0001*
	***R* (*P*-value)**	**Multiple linear correlation coefficient=0.866**
Survival time/Age	−0.239 (0.085)	*R* partial=0.0201; *P*-value=0.889
Survival time/Disease	−0.865 (<0.0001)*	*R* partial=−0.857; *P*-value<0.0001*
Survival time/Bacterial species	0.087 (0.538)	*R* partial=0.101; *P*-value=0.480

*Significant.

R, Pearson's linear correlation coefficient; *R* partial, the partial correlation coefficient is the coefficient of correlation of the variable with the dependent variable, adjusted for the effect of the other variables in the mode.

Pseudomonas spp. were significant negative predictors for PHC and positive predictors for CCA. In other words, the presence of *Pseudomonas* spp. and/or *E. coli* implicates not PHC but CCA. In particular, *Pseudomonas* spp. was the only significant positive predictor for GBC. Conversely, *Klebsiella* spp. was a positive predictor for PHC and a negative predictor for GBC, i.e. the presence of *Klebsiella* spp. implicates PHC but does not link to GBC. The results of univariate analysis showed that an increasing/decreasing presence of *E. coli* implicates an increasing/decreasing rate of CCA and a decreasing/increasing rate of PHC, respectively. With regard to *Klebsiella* spp., an increasing/decreasing presence of this microorganism implicates an increasing/decreasing rate of CCA and a decreasing/increasing rate of GBC, respectively. Finally, with regard to *Pseudomonas* spp. an increasing/decreasing rate implicates an increasing/decreasing rate for CCA and a decreasing/increasing rate for PHC and GBC, respectively.

DISCUSSION

In our study we found that *E. coli* is the microorganism involved not only with colonorectal cancer but also with neoplasms of the biliary tree [31]. *E. coli* has a well-defined role as a trigger in inflammatory and neoplastic processes and has been associated with the autophagy-inflammasome complex [2, 16, 17, 19, 32, 33]. Thus, *E. coli* may also represent a risk factor for neoplastic diseases of the biliary tree and pancreas. *Pseudomonas* spp. has also captured attracted research interest, demonstrating a contributory role in apoptosis and autophagy [2, 34]. The ambiguous interaction of *Pseudomonas* spp. with the nuclear factor κB (NF-κB) pathway, which is ineluctably associated with cancer, is still under investigation [35]. The identification of *E. coli* and *Pseudomonas* spp. may also have implications for therapy and, despite remarkable similarities between the two bacterial species, there are crucial differences that alter their resistance to b-lactams, fluoroquinolones and aminoglycosides [36].

Table 3. Univariate and multivariate linear correlation analysis between disease type and most frequently identified bacteria

Parameter	Univariate analysis	Multivariate analysis
	***R* (*P*-value)**	**Multiple linear correlation coefficient=0.896**
PHC/*E. coli*	−0.709 (<0.0001)*	*R* partial=−0.737; *P*-value=0.0001*
PHC/*Klebsiella* spp.	−0.160 (0.253)	*R* partial=0.289; *P*-value<0.0398*
PHC/*Pseudomonas* spp.	−0.747 (<0.0001)*	*R* partial=−0.776; *P*-value<0.0001*
	***R* (*P*-value)**	**Multiple linear correlation coefficient=0.831**
CCA/*E. coli*	0.678 (<0.0001)*	*R* partial=0.640; *P*-value<0.0001*
CCA/*Klebsiella* spp.	0.362 (0.0077)*	*R* partial=0.192; *P*-value=0.178
CCA/*Pseudomonas* spp.	0.676 (<0.0001)*	*R* partial=0.593; *P*-value<0.0001*
	***R* (*P*-value)**	**Multiple linear correlation coefficient=0.718**
GBC/*E. coli*	0.189 (0.183)	*R* partial=0.173; *P*-value=0.225
GBC/*Klebsiella* spp.	−0.503 (0.0001)*	*R* partial=−0.683; *P*-value<0.0001*
GBC/*Pseudomonas* spp.	0.291 (0.0342)*	*R* partial=0.529; *P*-value=0.0001*

*Significant.

R, Pearson's linear correlation coefficient; *R* partial, the partial correlation coefficient is the coefficient of correlation of the variable with the dependent variable, adjusted for the effect of the other variables in the mode.

In the last 30 years, GBC and PHC have shown a decreasing and an increasing trend, respectively, in mortality rates [37, 38]. GBC, specifically, in the Mediterranean area, was originally more prevalent than PHC mainly in subjects with prior cholelithiasis [39]. This decrease in GBC and cholelithiasis may suggest a change in the Mediterranean diet. In fact, there have been reports that Italy is experiencing decreased adherence to the Mediterranean diet with an increase in obesity, and this trend may have been in place since the 1990s coincidental with reduced physical activity and consumption of soda products [40, 41] with anti-carcinogenic properties [42–47]. In fact, GBC is more often associated with a calcium-rich diet while PHC is more often associated with a red meat diet, obesity and high glycaemic levels. A similar situation seems to be present in Brazil, with Alves confirming this tendency in South America [48] Advanced age remains a risk factor for PHC and remains a predictor of the risk of pancreatic cancer that is directly proportional to increasing age, as identified by several mathematical and epidemiological models [49–53].

Microbiota exerts diverse physiological functions including growth inhibition of pathogenic microorganisms, synthesis of compounds useful to the colonic mucosa, regulation of intestinal lymphoid tissue and synthesis of amino acids. Mucus seems to play an key role in protecting the intestinal mucosa and maintaining its integrity [20]. Changes in microbiota composition are mainly influenced by diet and age, as well as by genetic factors. Increasing evidence indicates that dysbiosis favours the production of genotoxins and metabolites associated with carcinogenesis, and the occurrence of intra-epthelial lymphocytosis of the gut [54]. Induced dysregulation of the immune response may promote and sustain inflammation in IBD, leading to carcinogenesis [39, 55].

The clinical goal of our work was to identify the composition of the microorganisms that colonize the biliary tract and cause biliary sepsis under favourable conditions. The presence of bactibilia is related to several variables that occur more often in patients who undergo elective cholecystectomy. *E. coli* and other Enterobacteriaceae, such as *Pseudomonas* spp., *Enterococcus* spp, and *K. pneumoniae* occur, especially if they coexist with *Candida* spp. colonization/infection. In the gut micro-environment, inflammation and oxidative stress may play a major role in carcinogenesis [16]. *In vivo* imaging could beeffective in gaining a better understanding of the effect of host–pathogen interactions on tumour development [56, 57]. The resistant pathogens described above were responsible for an outbreak in the Mediterranean area as reported by us and others [56–61]. Apart from *E. coli*, other pathogens, including *Fusobacterium* spp., *Streptococcus gallolyticus* and *Enterococcus faecalis* have recently been identified in patients affected with colonrectal cancer [61]. Genetic and phylogenetic analyses are required to verify the virulence of these well-known strains, especially in children. In our opinion, there is growing concern that abandoning the Mediterranean diet may be associated with a change in gut microflora and bactibilia.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
The study protocol conforms to the Declaration of Helsinki ethical guidelines for clinical studies and was approved by the local University Ethics Committee (University of Palermo, Institutional Review Board). All authors have approved the final version of the manuscript and adhere to the principles and Code of Conduct of Committee on Publication Ethics.

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