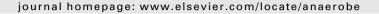
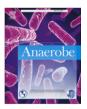
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Decrease in anaerobe-related bacteraemias and increase in *Bacteroides* species isolation rate from 1998 to 2007: A retrospective study

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ABSTRACT

Conflicting data have accumulated in recent years regarding the incidence of anaerobic bacteraemias. The aim of this study was to determine the prevalence of bacteraemias due to anaerobic bacteria and evaluate the importance of anaerobic blood cultures in a university hospital in Israel. A retrospective survey which focused on anaerobic blood culture bottles was performed on blood cultures received in our laboratory during the decade from January 1998 to December 2007. Anaerobic-related bacteraemias decreased during that period, whereas a significant increase was observed in *Bacteroides* species isolated from the blood cultures (from 18% during 1998–2002 to 43% during 2003–2007). Comparison of the medical records of 54 patients with *Bacteroides*-related bacteraemia during the two end periods (1998–1999 and 2006–2007) revealed a marked increase in complex underlying diseases. Hypertension and diabetes mellitus type II were found in 29% of the patients in 1998–1999 and increased to 43–45% of the patients in 2006–2007. Ischemic heart disease also increased from 14% of the patients in 1998–1999 to 43% in 2006–2007. We conclude that although positive anaerobic blood cultures account for a small percentage of positive blood samples, the growing involvement of *Bacteroides* species-related bacteraemias together with an increase in complex underlying diseases in these patients emphasize the importance of anaerobic blood cultures, particularly in patients with co-morbidities.

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1. Introduction

Bacteraemia due to anaerobic organisms occurs in 0.5–12% of blood cultures [1–3]. Recent studies from Europe and the USA presented inconsistent data regarding the prevalence of anaerobic bacteraemias between 1993 and 2006. Lassmann et al. and Blairon et al. reported a trend towards an increase in the incidence of anaerobic bacteraemias [4,5], whereas Fenner et al. demonstrated a decline in the incidence of bacteraemias due to anaerobic organisms [2]. Moreover, a study by Grohs et al. demonstrated no change in the anaerobic bacteraemia rate from 2001 to 2004 [6]. We examined the occurrence of bacteraemias due to anaerobic bacteria during a 10-year period, from January 1998 to December 2007, at the Assaf Harofeh Medical Center, in order to identify current trends of anaerobic bacteraemias in our institute.

Recent studies have addressed the clinical significance and outcome of anaerobic bacteraemias during shorter periods of 1–2 years [6-10], raising the question of the need for systematic use of anaerobic bottles. Grohs et al. pointed out that 13.7% of the patients had a positive blood culture detected solely by the anaerobic bottle, thus suggesting that sampling of aerobic bottles alone could lead to underestimated bacteraemia diagnoses [6]. Furthermore, Robert et al. demonstrated that anaerobes may be unexpectedly involved in some cases, making the use of anaerobic bottles essential [8]. On the other hand, Iwata and Takahashi demonstrated that 11.5% of the cases were detected by anaerobic blood cultures alone. However, they suggested that most of these cases could be predicted by clinical evaluation [9]. In this report we retrospectively analyzed the proportion of anaerobes out of total blood cultures and the distribution of different anaerobic bacteria isolated from positive anaerobic blood cultures in order to characterize their involvement in anaerobic blood cultures during a 10-year period.

Indeed, the incidence of anaerobic bacteria-related bacteraemia is low. However, bacteraemias remain associated with a high mortality rate of 14–60% [3–9,11] and are correlated with age, underlying diseases as well as appropriate antimicrobial treatment [5,8,11]. Since *Bacteroides* species are associated with disease

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severity [3], we analyzed blood samples that contained *Bacteroides* bacteria during the two periods of 1998–1999 and 2006–2007 in order to determine whether these pathogens were related to mortality rate, age and underlying diseases.

2. Patients and methods

Assaf Harofeh Medical Center (Israel) is a 928-bed, university-affiliated, primary and tertiary care teaching hospital, serving an urban and rural population of 440,000 people. Clinical data was retrieved from patients' medical records. Bacteraemias were considered clinically relevant when a patient had a leukocyte count greater than 11,000, temperature higher than 38 °C, or clinical evidence consistent with infection.

Two bottles were routinely collected for blood culture: an aerobic bottle enabling preferential growth of aerobic and facultative anaerobic microorganisms, and an anaerobic bottle enabling preferential growth of strict anaerobic bacteria. All blood culture bottles were placed in a BacT/Alert system at 37 °C, incubated up to one week and monitored in accordance with the manufacturer's instructions. Briefly, the automated BacT/Alert (bioMérieux, Lyon, France) system was used for the incubation and detection of positive blood cultures. The plastic bottles contain FAN medium with charcoal and Fuller's earth that adsorb antibiotics present in blood samples and allow the recovery of microorganisms [12–14].

In this study, only BacT/Alert positive anaerobic bottles in which the presence of bacteria was indicated by Gram stain and which showed no growth of bacteria under aerobic conditions were further analyzed. These samples were systematically plated on BA-139 anaerobic blood agar with amikacin (Novamed Ltd., Jerusalem, Israel). Bacterial identification was performed according to the Wadsworth-KTL Anaerobic Bacteriology Manual [15]. Final identification was performed using the API 32A kit (bioMérieux, Lyon, France).

Table 1 presents the prevalence of anaerobic isolates of Gramnegative bacilli, Gram-positive bacilli and Gram-positive cocci. It should be noted that since clinical data was not available for the entire study period, determination of sepsis versus contaminants was not possible for each isolate.

Comparison of bacteraemia rates between two time periods, i.e. 1997–2002 and 2003–2007, was performed by the Student t-test analysis. The p values are two-sided. The results were considered statistically significant when $p \le 0.05$.

3. Results

The data in this section are presented either as a summary of each year from 1998 until 2007, or as a comparison between two 5 year periods, i.e. 1998–2002 and 2003–2007, thus enabling statistical analyses. Analysis of patients' medical records was performed on both ends of the study period, i.e. during 1998–1999 and 2006–2007, since medical data were not available for the entire study period.

The total anaerobic blood samples detected by BacT/Alert as positive during the period from 1998 to 2007 were 158 \pm 9 per year out of 202,292 anaerobic blood cultures bottles, corresponding to a mean of 0.78%. All of these samples were examined for the presence of bacteria by light microscopy and showed no aerobic bacterial growth.

The mean number of total collected anaerobic bottles remained unchanged between 1998–2002 and 2003–2007 (19293 \pm 1373 and 21165 \pm 1714, respectively, p=0.09). However, the number of positive anaerobic blood cultures indicated by BacT/Alert decreased from 0.84% during 1998–2002 to 0.74% during 2003–2007 (p=0.03).

Analysis of the percentage of isolated bacteria out of the total positive samples indicated that isolated bacteria were highest in 2001–2002 compared to the other years and exceeded 80% of the total positive samples (Fig. 1). However, analysis of these isolates clearly showed that nearly half of the isolated bacteria were *Propionibacterium* spp. The mean isolation rate after exclusion of *Propionibacterium* spp. was $30\% \pm 9$ of the positive samples, suggesting similar rates of anaerobic bacteria isolated from blood samples during this 10-year period (Fig. 1).

All 472 anaerobic bacteria species and sub-species isolated in our laboratory (excluding *Propionibacterium* spp.) during 1998–2007 are summarized in Table 1, where they are divided into Gram-negative bacilli such as *Bacteroides* spp., *Prevotella* spp. and *Fusobacterium* spp.; Gram-positive bacilli, namely *Clostridium* spp.; and Gram-positive cocci including *Streptococcus* spp. and *Peptostreptococcus* spp. Other anaerobic bacteria not detailed in Table 1 include Gram-negative cocci *Veilonella* spp., Gram-positive *Eubacterium* spp. and *Staphylococcus sacharolytica*.

As expected, the most abundant anaerobic species isolated throughout these 10 years were *Bacteroides* species, which comprised 31.2% of the anaerobes, on average, followed by *Clostridium* spp. (14.4%), *Streptococcus* spp. and *Peptostreptococcus* spp. (6.9% and 3.4%, respectively), *Prevotella* spp. (2.6%) and *Fusobacterium* spp. (2.2%).

Further analysis of the anaerobic organisms isolated from blood cultures revealed that the percentage of Gram-negative bacilli increased markedly between the two study periods (Fig. 2). *Prevotella* spp. increased by 6-fold, from 0.7% during 1998–2002 to 4.5% during 2003–2007 (p=0.05). The *Bacteroides fragilis* group increased from 18.1% during 1998–2002 to 44.3% during 2003–2007 (p=0.02). This significant increase reflects a trend of annual increases (data not shown). *Fusobacterium* spp. isolation also increased. However, this increase was not statistically significant. The same trend was observed for *Clostridium* spp. However, Gram-positive cocci remained unchanged (Fig. 2).

Since Bacteroides spp. were the most abundant species related to bacteraemias (Table 1), and a significant increase in their isolation from positive blood cultures was evident (Fig. 1), we further characterized the cases in which patients developed Bacteroides-related bacteraemias. Medical records of 54 patients were studied: the records of 14 patients admitted in 1998-1999 and 40 patients admitted in 2006-2007 were retrieved. Clinical data including underlying diseases, antibacterial treatment and hospitalization outcome are summarized in Table 2. It should be noted that only two patients underwent surgical procedures during 1998-1999 and 3 during 2006–2007. Most of the patients were suspected of having sepsis/septic shock upon arrival at the hospital (data not shown). As shown in Table 2, the number of males and females were identical (n = 27 each) during these two periods. However, the male/female ratio decreased from 1.8 in 1998-1999 to 0.8 in 2006-2007. The patients' mean ages were similar: 63 ± 22 and 70 ± 18 years. Empirical anti-anaerobe treatment, usually Metronidazole, was applied in 50-55% of the cases and the mortality rate was 21-30%.

Analysis of the underlying diseases at the two end periods indicated that the leading underlying diseases were hypertension (HTN) and diabetes mellitus type II (DM type II). However, during 1998–1999 twenty-nine percent of the patients had HTN and DM type II, whereas during 2006–2007 the percentage of these diseases increased to 45% and 43%, respectively. It should be noted that during 1998–1999 an equal distribution of underlying diseases was found between HTN, DM type II (29%), chronic renal failure (CRF), diverticulosis and carcinoma (21%). However, during 2006–2007 the main underlying diseases were HTN, DM type II and ischemic heart disease (43–45%). CRF remained unchanged (23%), diverticulosis decreased from 21% to 3%, and carcinoma from 21% to

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