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Antimicrobial susceptibility of anaerobic bacteria

Genetic identification and antimicrobial susceptibility of clinically isolated anaerobic bacteria: A prospective multicenter surveillance study in Japan

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ABSTRACT

This prospective multicenter surveillance study was designed to provide antimicrobial susceptibility profiles of clinical anaerobic bacteria with genetic species identification in Japan. In 2014, a total of 526 non-duplicate clinical anaerobic isolates were collected from 11 acute-care hospitals in the Kyoto and Shiga regions of Japan. Genetic identification was performed using 16S rRNA sequencing. Minimum inhibitory concentrations were determined in the central laboratory and were interpreted using the CLSI criteria. Genetic analysis provided species-level identification for 496 isolates (83 species in 40 genera) and genus-level identification for 21 isolates (13 genera). Among these 517 isolates, the most frequent anaerobes were *Bacteroides* spp. (n = 207), *Prevotella* spp. (n = 43), *Clostridium* spp. (n = 40), and *Peptoniphilus* spp. (n = 40). *B. fragilis* was the most common species (n = 107) and showed 91.6%–97.2% susceptibility to β-lactam/β-lactamase inhibitor combinations (BLBLIs; ampicillin-sulbactam, amoxicillin-clavulanate, and piperacillin-tazobactam) and carbapenems (imipenem and meropenem) as well as 100% susceptibility to metronidazole. Gram-negative anaerobes were highly susceptible to metronidazole (99.0%) followed by BLBLIs and carbapenems (>90% each). BLBLIs or carbapenems also retained activity against Gram-positive anaerobes (99.5%–100%) except *Clostridioides difficile*. All isolates were susceptible to combinations of metronidazole with BLBLIs or carbapenems. Thus, BLBLIs or carbapenems are first choices for empirical therapy of anaerobic infections in Japan, and these antimicrobials in combination with metronidazole should be reserved for very severe infections and targeted therapy.

1. Introduction

Anaerobic bacteria have been implicated in severe human infections and bacteremia. These organisms are commonly found in polymicrobial aerobic or anaerobic infections of endogenous origin, including oral, intra-abdominal, pulmonary, gynecological, skin and soft tissue infections. Most clinical microbiology laboratories in Japanese hospitals perform limited anaerobic bacteriology. Indeed, susceptibility testing is not routinely performed because for anaerobic cultures (isolation and identification of anaerobic bacteria), it is time-consuming and expensive and has some technical difficulties. While some new automated identification methods, such as phenotypic methods and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS), are gaining popularity, the accuracies of these methods are still limited [1–3]. Genetic identification has been considered the “gold standard” identification method and provides the most reliable identification for anaerobic bacteria [4,5].

In recent years, antimicrobial resistance among anaerobic bacteria, especially *Bacteroides* spp., has increased worldwide among antimicrobials that have been considered universally active, such as carbapenems and nitroimidazoles [6–11]. Some case reports have

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demonstrated severe infectious diseases caused by *Bacteroides fragilis* resistant to both carbapenems and metronidazole [12,13]. Antimicrobial susceptibility surveillance data are important to treat infectious diseases caused by anaerobic bacteria because inactive therapy is directly linked to poor clinical outcomes and the activity of antimicrobials varies by region [8,14]. However, few comprehensive antimicrobial susceptibility surveillance data for anaerobic bacteria are available in Japan [3].

Therefore, it is important to provide updated antimicrobial susceptibility surveillance data with accurate identification to better understand the epidemiology of anaerobes and to guide physicians in performing appropriate empirical antimicrobial therapy. This prospective multicenter study was designed to collect recent data on the prevalence of frequently isolated anaerobic bacteria with genetic identification as well as data on the susceptibility of clinically significant anaerobic bacteria in Japan.

2. Materials and methods

2.1. Bacterial isolates

From June to December 2014, all clinical anaerobic isolates were prospectively stored. A total of 526 non-duplicate clinical anaerobic isolates were collected from 11 acute-care hospitals in the Kyoto and Shiga regions of Japan. These samples were isolated from the following clinical specimens: pus/wound/aspirated fluid, 186 (35%); blood, 120 (23%); abdominal sites, 101 (19%); gynecological sites, 54 (10%); urine, 20 (3.8%); oropharyngeal, 17 (3.2%); stool, 17 (3.2%); respiratory tract, 7 (1.3%); and other sites, 4 (0.8%). The isolates were stored in 20% skimmed milk at −80 °C at each hospital, and they were then subcultured and examined at the reference laboratory at Kyoto University.

2.2. Species identification by 16S rRNA gene analysis

The 16S rRNA gene was amplified by PCR using the 27f and 1492r primer pair [15], and the amplicons were directly sequenced. Species identification was performed using SILVA small subunit rRNA database release 128 [16] and BLAST+ [17]. A >98.7% identity and 97–98.7% identity were the criteria used for species-level and genus-level identification, respectively [18].

2.3. Antimicrobial susceptibility testing

The minimum inhibitory concentrations (MICs) of the following antimicrobial agents were tested: ampicillin, piperacillin, ampicillin-sulbactam, amoxicillin-clavulanate, piperacillin-tazobactam, cefotaxime, cefoperazone-sulbactam, cefoxitin, cefmetazole, flomoxef, imipenem, meropenem, moxifloxacin, chloramphenicol, clindamycin, vancomycin, teicoplanin, linezolid and metronidazole. The testing was performed with a broth microdilution method using Dry Plates Eiken (Eiken, Tokyo, Japan) following the CLSI guideline [19]. Briefly, 10^6 CFU/mL bacteria were inoculated in Brucella broth with 5% lysed horse blood, 1 μg/mL vitamin K_1 and 5 μg/mL hemin and incubated anaerobically at 35 °C for 48 h. For testing metronidazole, we used the Etest gradient strip (bioMérieux, Marcy l'Etoile, France) on Brucella agar with 5% laked sheep blood, vitamin K and hemin because Dry Plates Eiken does not include metronidazole. *B. fragilis* ATCC 25285, *Bacteroides thetaiotaomicron* ATCC 29741, and *Eggerthella lenta* ATCC 43055 were used in each testing run for quality control of the susceptibility tests. The isolates were categorized by CLSI [19] and EUCAST breakpoints [20]. The results and discussion will be described based on the CLSI breakpoints unless otherwise stated.

2.4. Statistical analysis

The categorical variables were compared using Fisher's exact tests. The concordance of the susceptibility testing results between the CLSI and EUCAST breakpoints was tested using the exact form of McNemar's test. The continuous variables were compared using the Wilcoxon signed-rank test. A p-value < 0.05 was considered statistically significant. We conducted our statistical analysis using Stata, version 13.1 (StataCorp, College Station, TX, USA).

3. Results and discussion

3.1. Species identification

Genetic analysis provided species-level identification for 496 isolates (94.3%; 83 species in 40 genera), genus-level identification for 21 isolates (13 genera), and no identification for 9 isolates. The most commonly isolated genera were *Bacteroides* (n = 207), *Prevotella* (n = 43), and *Fusobacterium* (n = 12) among Gram-negative anaerobes and *Clostridium* (n = 40), *Peptoniphilus* (n = 40), and *Finegoldia* (n = 21) among Gram-positive anaerobes. *B. fragilis* was the most commonly isolated species (n = 107), followed by *B. thetaiotaomicron* (n = 37), and *Peptoniphilus asaccharolyticus/harei* (n = 35) (Table 1 and Dataset 1). The genus or species distribution of these study isolates was in agreement with recent surveillance studies [9–11], except for a larger number of *Bacteroides and Parabacteroides* spp. isolates in the present study (42%) compared to those from Canada and Croatia (29% each) [9,10].

3.2. Bacteroides and Parabacteroides spp.

The results of antimicrobial susceptibility testing for Gram-negative anaerobes are shown in Table 2. *B. fragilis* showed 91.6%–97.2% susceptibility to β-lactam/β-lactamase inhibitor combinations (BLBLIs, i.e., ampicillin-sulbactam, amoxicillin-clavulanate, and piperacillin-tazobactam) and carbapenems, >99% susceptibility to metronidazole and chloramphenicol, but lower susceptibility to moxifloxacin (79.4%) and clindamycin (63.6%). *Bacteroides* and *Parabacteroides* species without *B. fragilis* (non-*B. fragilis*) showed 86.4%–98.2% susceptibility to BLBLIs and >98% susceptibility to carbapenems and metronidazole. Compared with *B. fragilis*, non-*B. fragilis* showed a higher susceptibility to meropenem ($p = 0.02$; exact McNemar's test) and a lower susceptibility to cefmetazole and clindamycin ($p < 0.001$ and 0.02, respectively; exact McNemar's test).

Similar to recent reports [8–11,21,22], BLBLIs demonstrated good activity against *Bacteroides* and *Parabacteroides* spp. Taiwanese reports [6,23] described lower susceptibility rates of both *B. fragilis* and non-*B. fragilis* to ampicillin-sulbactam and amoxicillin-clavulanate (45%–65%), although these rates are unusual, as most other previous studies reported 80–100% susceptibilities of BLBLIs for *Bacteroides* spp. According to the cumulative antimicrobial susceptibility report in USA hospitals shown in the CLSI document [19], *Parabacteroides distasonis* demonstrated the lowest susceptibility rate of BLBLIs (56–66%) among the *Bacteroides* group. Six *P. distasonis* isolates in this study showed similar susceptibility rates of BLBLIs (50%–83%). In contrast, carbapenems showed good activity against *Bacteroides* spp., especially to non-*B. fragilis*. The CLSI data also indicate ≥95% susceptibility rates for all noted *Bacteroides* spp. The susceptibility rates of *Bacteroides* spp. to clindamycin was as low as 40–60% in this study and in other reports [9–11], which limits its utility in clinical settings.