Antimicrobial susceptibility phenotypic patterns and genotypic analysis of anaerobes isolated from healty people

メタデータ	言語: English 出版者: 公開日: 2022-06-22 キーワード (Ja): キーワード (En): 作成者: Vu Thi My Hanh メールアドレス: 所属:
URL	http://hdl.handle.net/20.500.12099/88137

氏名（国籍）	Vu Thi My Hanh（ベトナム）
学位の種類	博　士　（医科学）
学位授与番号	甲第　６３　号
学位授与日付	令和　４　年　３　月　２５　日
専攻	医療情報学専攻
学位論文題目	健康人から分離された嫌気性菌の抗菌薬感受性パターンと遺伝子型解析 （Antimicrobial susceptibility phenotypic patterns and genotypic analysis of anaerobes isolated from healthy people）
学位論文審査委員	（主査）教　授　　山本　眞由美 （副査）教　授　　森田　洋子 （副査）教　授　　田中　香お里

論文内容の要旨

Introduction: Anaerobic bacteria, including the most frequently isolated Bacteroides fragilis group (BFG) strains, are the normal resident gastrointestinal microbiota members. BFG is human opportunistic pathogens that cause severe intra-abdominal infections, postoperative wounds, skin, and soft tissue infections, together with aerobic bacteria. The number of antimicrobials effective against BFG bacteria is relatively limited. Increasing antimicrobial agent resistance in BFG strains has been reported in recent years and observed worldwide, including resistance to β-lactams, tetracyclines, macrolides, clindamycin, and fluoroquinolones. In literature, BFG is in the spotlight as an important clinical pathogenic anaerobic, but not so much available research on BFG from healthy people in different geographical areas.

Objectives: This dissertation conducted a study on BFG isolated from healthy individuals in Vietnam and Japan with four specific objectives as follows:

1. To investigate the major species of Bacteroides spp. in the microbiota of healthy individuals in Vietnam and Japan
2. To investigate the phenotypic and genotypic patterns of antimicrobial-resistance in Bacteroides fragilis group isolated from healthy individuals in Vietnam and Japan
3. To establish and validate a protocol from sample collection to BFG complete genome sequencing, control and assure genome sequences quality, and register to public databases.
4. To make whole-genome sequencing of meropenem–resistant Bacteroides for further analysis of carbapenem-resistant mechanisms

Methods: BFG was isolated from fecal samples of healthy individuals in Vietnam and Japan. Isolated strains were identified using MALDI-TOF MS, and the minimum inhibitory concentration (MIC) of 18 antibiotics was determined using the agar dilution method. Additionally, 20 antimicrobial resistance genes were detected using standard PCR. Whole-genome sequences of chosen strains were obtained by using a hybrid approach, combining Nanopore long-read sequencing and DNBseq short-read sequencing on the same qualified high molecular weight DNA. Genome sequences were obtained deposited at the DNA Data Bank of Japan (DDBJ) according to DDBJ, annotated by using the DDBJ Fast Annotation and Submission Tool (DFAST). Resistance genes were determined by ResFinder v4.0 and AMRFinderPlus.

Results:

1. A total of 139 BFG strains belonging to 11 BFG species were isolated from the two countries. B. fragilis was not the predominant species in either country, but the difference in the prevalence of B. fragilis between Vietnam and Japan was significant. The first, second, and third most common species were Parabacteroides distasonis (35.5%), Bacteroides thetaiotaomicron (19.7%), and Phocaeicola (former Bacteroides) vulgatus (17.1%) in Vietnam, and Bacteroides ovatus (23.8%), P. vulgatus (22.2%), and P. distasonis (15.9%) in Japan.
2. Isolations from Vietnam and Japan showed some similarities in terms of MIC50 values, MIC90 values, and percentages of resistant strains. However, isolations from Vietnam showed significantly higher resistance to piperacillin, cefmetazole, clindamycin, tetracycline, and minocycline. ErmB, tet36, tetM, nim, catA, and qnrA were not found in either country. CepA was more common in B. fragilis than in non-fragilis Bacteroides. In contrast, cfiA, ermG, mefA, msrSA, tetX, tetX1, bexA, qnrB, and qnrS were found only in non-fragilis Bacteroides. ermG, mefA, and msrSA were statistically higher prevalence in Japan compared to Vietnam. In contrast, linA and qnrS were higher prevalence in Vietnam compared to Japan.
3. A protocol for whole-genome sequencing of BFG genome from sample collection to complete genome sequences, control and assure genome sequences quality and register to public databases was established and validated. The protocol was applied to analyzing genomes of three P. vulgatus isolated from a healthy individual in Japan in the scope of antimicrobial resistance. The whole-genome sequencing approach showed multiple advantages, not only in determining resistance genes but also locating them for further analysis on resistance mechanism and horizontal gene transfer. This work also contributed to the public genome database complete genomes of three P. vulgatus, that are not yet well-established.
4. Two meropenem-resistant BFG from Vietnam and Japan were sequenced and analyzed by the protocol mentioned above this protocol. Whole-genome analysis revealed multiple determinants for the multidrug-resistant phenotype of those strains that could not be fulfilled by other investigation methods such as standard PCR. Questions still remain about the meropenem-resistance mechanism since there were not any known carbapenem-resistance genes found.

Conclusion: This study is the first report on the antimicrobial susceptibility patterns in the BFG isolated from healthy individuals in Vietnam and Japan. BFG isolated from healthy people in Vietnam and Japan carried various types of antimicrobial-resistant genes and showed diversities in phenotypic resistant patterns. The distribution of various antimicrobial agent resistance genes also differed between the two countries. The whole-genome approach has many advantages and is beneficial for multi-drug resistant BFG for genome analysis. The development of recent technology in bioinformatic supports to have a better understanding of BFG genomes but still faces challenges due to the plasticity of BFG genomes.

Considerations and suggestions for future research: Antimicrobial resistance is a big concern in BFG isolated from healthy people in both Vietnam and Japan. With that high prevalence of resistance genes, BFG is a pool that can harm the host in many ways, as discussed. The horizontal gene transfer in BFG was presented and discussed in many publications, that very interesting to investigate in our samples.

論文審査結果の要旨

Bacteroides 属とこの属の再編成で新設された Parabacteorides 属、Phocaeicola 属は、ヒト腸内細菌叢の主要構成菌群であると同時に、腹腔内感染症、術後創感染、皮膚軟部組織感染症などの内因性感染症から通性嫌気性菌とともに高頻度に分離される他、一部の菌種は菌血症からも分離され、臨床上重要な嫌気性菌として、Bacteroides fragilis group (以下 BFG)と呼ばれている。嫌気性菌感染症から最も高頻度に分離される菌群である。BFG に有効な抗菌薬は限られており、近年、βラクタム系、テトラサイクリン系、マクロライド系、クリンダマイシン、キノロン系に対する耐性菌の増加が、世界的に報告されてきている。これまで、臨床分離の BFG については、多数の抗菌薬感受性・耐性の報告があり、薬剤耐性遺伝子の分布についても、少なからず報告がある。しかしながら、これら臨床分離株のソースである腸内細菌叢について、抗菌薬感受性・耐性や薬剤耐性遺伝子の保有状況を検討した報告は希であり、ベトナム人および日本人についての情報は皆無である。ベトナムの健康成人の便から分離された大腸菌については、日本人には見られないコリスチン耐性遺伝子を高頻度に保有していることが報告されている。本論文では、腸内細菌叢の主要構成菌群である BFG を対象に、両国の健康成人の便からの分離菌について、1. 主要構成菌種の比較、2. 抗菌薬感受性・耐性プロファイルおよび耐性遺伝子プロファイルの検討、3. BFG の全ゲノム解析のための方法の検証とデータベース登録、4. カルバペネム耐性株の耐性機構解明のための全ゲノム解析を行った。これらの検討結果から、1. 両国とも上位の優勢菌種の構成については似ていること、2. 感受性・耐性のパターンも似ているが、piperacillin, cefmetazole, clindamycin, tetracycline, and minocycline の耐性率がベトナムにおいて有意に高いこと、今回検索した耐性遺伝子だけでは説明できない耐性株が存在すること、3 については、BFG のサンプル調製、解析とも容易ではないが、一定の方法を確立できたこと、全ゲノム解析は、耐性機構の解明に有用である事が示唆されたこと、4 については、本検討では既知のカルバペネム耐性遺伝子は見つからなかったため、関連しうる遺伝子のさらなる解析が必要である事が示された。

本研究では、今後の課題が多く残されているが、嫌気性菌の薬剤耐性機構に関する研究においては、未開拓の領域に踏み込んだ挑戦的なアプローチであり、情報が極めて不足しているこの領域において、最初の足場となる研究である。このような観点から、申請者 Vu Thi My Hanh 氏の論文は、学術的価値が極めて高く、審査の結果、学位論文に値するものと判定した。

最終試験結果の要旨

Vu Thi My Hanh 氏の学位論文の主要部分は、審査付き学術雑誌に公表済みの二編の論文に基づくも

のであり、本論文が学位論文として､完成された内容である事を確認した。

　また、公聴会において、学位論文の内容に関する事項、すなわち、本研究において検討したベトナムと日本の健康成人からの菌の分離･同定、各種抗菌薬に対する薬剤感受性･耐性プロファイルおよび薬剤耐性遺伝子の分布と両国間の比較、分離株の一部について実施した全ゲノム解析、今後の研究の展開や将来性などについて諮問を行った。申請者からは十分な内容の回答を得られたので、博士（医科学）の学位に適するものと判断し、最終試験に合格と判定した。

論文リスト

1．Hanh Vu, Masahiro Hayashi, Thang Nam Nguyen, Diep Thi Kong, Hoa Thi Tran, Yoshimasa Yamamoto, Kaori Tanaka, Comprison of phenotypic and genotypic patterns of antimicrobial-resistant Bacteroides fragilis group isolated from healthy individual in Vietnam and Japan. Infection and Drug Resistance. 14: 5313～5323. (2021) 【CS:3.9】

2. Hanh Vu, Yoshinori Muto, Masahiro Hayashi, Hideki Noguchi, Kaori Tanaka, Yoshimasa Yamamoto, Complete genome sequences of three Phocaeicola vulgatus strains isolated from a healthy Japanese individual. Microbiology Resource Announcements. in press 【CS:1.5】