様式第１９号

学位論文審査の結果の要旨

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論文題目

Discovery of novel mutations for clarithromycin resistance in *Helicobacter pylori* by using next-generation sequencing

（次世代シーケンサーを用いたヘリコバクター・ピロリのクラリスロマイシン耐性に関与する新規変異の発見

論文掲載雑誌名

Journal of Antimicrobial Chemotherapy

論文要旨

Objectives: Resistance to clarithromycin is the most important factor causing failure of *Helicobacter pylori* eradication. Although clarithromycin resistance is mainly associated with three point mutations in the 23S rRNA genes, it is unclear whether other mutations are associated with this resistance.

Methods: Two types of clarithromycin-resistant strains (low- and high-resistance strains) were obtained from clarithromycin-susceptible H. pylori following exposure to low clarithromycin concentrations. The genome sequences were determined with a next-generation sequencer. Natural transformation was used to introduce the candidate mutations into strain 26695. Etest and an agar dilution method were used to determine the MICs.

Results: High-resistance strains contained the mutation A2143G in the 23S rRNA genes, whereas low-resistance strains did not. There were seven candidate mutations in six genes outside of the 23S rRNA genes. The mutated sequences in hp1048 (infB), hp1314 (rpl22) and the 23S rRNA gene were successfully transformed into strain 26695 and the transformants showed an increased MIC of and low resistance to clarithromycin. The transformants containing a single mutation in infB orrpl22 (either a 9 bp insertion or a 3 bp deletion) or the 23S rRNA gene showed low MICs (0.5, 2.0, 4.0 and 32 mg/L, respectively) while the transformants containing double mutations (mutation in the 23S rRNA genes and mutation in infB or rpl22) showed higher MICs (>256 mg/L).

Conclusions: Next-generation sequencing can be a useful tool for screening mutations related to drug resistance. We discovered novel mutations related to clarithromycin resistance in H. pylori (infB and rpl22), which have synergic effects with 23S rRNA resulting in higher MICs.

以上の発表内容を審査委員で合議し，本論文は学位論文に値すると判断した。

様式第２０号

最終試験
の結果の要旨
~~学力の確認~~

審査区分 ㊀・論	第５３２号	氏名	とらん たん びん Tran Thanh Binh
審査委員会委員		主査氏名	村上和成
		副査氏名	種久泰生
		副査氏名	江下優樹

1. What kind of drug is used for clarithromycin-resistant strains. Is the MIC >1mg standard value?
2.クラリスロマイシン耐性のヘリコバクター・ピロリについて検討されているが、メトロニダゾール耐性株についてはどうか。
3. How about the prevalence of drug resistances in H. pylori in the world?
4. Please explain how CLA resistant strains affect eradication failure.
5. More than 90% of CLA-resistant strains have up to point mutations in the region of domain five of 23S rRNA. But the goal in this study is to clarify the gene mutations outside the domain five using newer sequencer. Should the goal be the mutation of domain five?
6. 感受性ヘリコバクター・ピロリからクラリスロマイシン耐性株を得るためのスクリーニング法について。
7. クラリスロマイシン耐性のヘリコバクター・ピロリは、クラリスロマイシンなしの環境で培養し続けると感受性株に戻ることがあるのか。
8. You constructed CLA-resistant strains as described previously. Reference No.33 could be how to induct metronidazole resistance in vitro?
9. Were there any difference of mutations associated with CLA-resistances between constructed strains and clinical isolated strains?
10. ヘリコバクター・ピロリの遺伝子数は1,500程といわれるが、次世代シーケンサーで調べた感受性株と抵抗性株で異なる塩基の数について。
11. 次世代シーケンサーで読み込まれたリード数が、感受性標準株のゲノム上にマッピングされた割合について。
12. 26695-1CH show high MIC (over 256), but the strains with natural transformation show low level of resistances. Could you explain this finding?
13. Only 23SrRNA mutation shows MIC32, and added rpl22 or infB mutation show very high MIC? Are these combinations of mutations occur spontaneously?
14. How frequently is the novel mutations found in isolated strains from real patients in Vietnam?
15. Speaking about cancers, when low-grade tumors progress to high-grade ones, usually mutations of high-grade ones include those of low-grade ones. In this experiment, mutations of high-resistant strains donnot necessarily include those of low-resistant ones. Are such events common in bacteria?
16. ヘリコバクター・ピロリ感染の患者から、今回新たに得た抵抗性株および、従来から知られている抵抗性株の両特性を持つようなヘリコバクター・ピロリが患者から得られる可能性について。Do you think spontaneous mutations were rare?
17. Could you explain the synergic relationship of gene mutations related to CLA-resistance?
18. These two mutations may cause co-effect with 2143G involved in increased resistance to CLA.
19. What is the usefulness of the next generation sequencer in the future?

これらの質疑に対して，申請者は概ね適切に回答した。よって審査委員の合議の結果，申請者は学位取得有資格者と認定した。

（注）不要の文字は２本線で抹消すること。

様式第８号 No. 1

学　位　論　文　要　旨

氏名　Tran Thanh Binh

論　文　題　目

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（次世代シーケンサーを用いたヘリコバクター・ピロリのクラリスロマイシン耐性に関与する新規変異の発見）

要　　旨

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Methods Two types of clarithromycin-resistant strains (low- and high-resistance strains) were obtained from clarithromycin-susceptible *H. pylori* following exposure to low clarithromycin concentrations. The genome sequences were determined with a next-generation sequencer. Natural transformation was used to introduce the candidate mutations into strain 26695. Etest and an agar dilution method were used to determine the MICs.

Results High-resistance strains contained the mutation A2143G in the 23S rRNA genes, whereas low-resistance strains did not. There were seven candidate mutations in six genes outside of the 23S rRNA genes. The mutated sequences in *hp1048* (*infB*), *hp1314* (*rpl22*) and the 23S rRNA gene were successfully transformed into strain 26695 and the transformants showed an increased MIC of and low resistance to clarithromycin. The transformants containing a single mutation in *infB* or *rpl22* (either a 9 bp insertion or a 3 bp deletion) or the 23S rRNA gene showed low MICs (0.5, 2.0, 4.0 and 32 mg/L, respectively)

while the transformants containing double mutations (mutation in the 23S rRNA genes and mutation in *infB* or *rplV*) showed higher MICs (>256 mg/L).

Conclusions Next-generation sequencing can be a useful tool for screening mutations related to drug resistance. We discovered novel mutations related to clarithromycin resistance in *H. pylori* (*infB* and *rplV*), which have synergic effects with 23S rRNA resulting in higher MICs.