

論文審査の結果の要旨

博士の専攻分野の名称	博士（歯学）	氏名	LE Nguyen Tra Mi
学位授与の条件	学位規則第4条第1・2項該当		
論文題目 Oral colonisation by antimicrobial-resistant Gram-negative bacteria among long-term care facility residents: prevalence, risk factors, and molecular epidemiology (長期療養型施設入所者における口腔内薬剤耐性グラム陰性菌の保有率、リスク因子及び分子疫学の検討)			
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〔論文審査の結果の要旨〕			
<p>Nursing and health-care associated pneumonia (NHCAP) and aspiration pneumonia are among the major causes of mortality among the elderly in Japan. Various pathogens have been known to associated with aspiration pneumonia, for examples, <i>Streptococcus pneumoniae</i>, <i>Staphylococcus aureus</i>, <i>Enterobacteriaceae</i>, and Oral streptococci. Especially, when the patients have a risk factor for the involvement by drug-resistant pathogens such as history of antimicrobial chemotherapy, additional pathogens may be involved, such as <i>Pseudomonas aeruginosa</i>, <i>Acinetobacter</i>, or ESBL-producing <i>Enterobacteriaceae</i> and MRSA. The student aimed at investigating the prevalence and molecular epidemiology of antimicrobial-resistant (AMR) Gram-negative bacteria in the oral cavity of long-term care facility (LTCF) residents, and to analyse the risk factors for such carriers.</p> <p>The study was carried out in 98 residents of an LTCF in Hiroshima City. The residents age was between 55 and 101 years old. Oropharyngeal swabs were collected and plated on screening media for ESBL-producing (CHROMagar™ ESBL) and carbapenem-resistant bacteria (CHROMagar™ mSuperCARBA). Isolates were identified and tested for antibiotic susceptibility using the Vitek-2 system and the results were interpreted according to the Clinical Laboratory Standards Institute M100-S25. Accordingly, 54 isolates from 37 patients (38%) were detected as ARB using screening agar plates and primarily belonged to three main genera: <i>Acinetobacter</i> spp. (35%), <i>Enterobacteriaceae</i> spp. (22%), and <i>Pseudomonas</i> spp. (19%).</p> <p>Next, she performed PCR to identify the epidemic clones, used whole-genome sequences (WGS) to detect AMR genes, sequence types (ST), and whole-genome comparisons, and conducted biofilm assay to evaluate the biofilm formation ability of the isolates. She detected 4 <i>Escherichia coli</i> isolates that were resistant to various β-lactams and ciprofloxacin, belonged to ST131, serotype O25:H4, <i>fimH30</i>, and carried multiple plasmid-mediated AMR genes. Besides, these isolates also carried mutations in DNA sequences resulting in fluoroquinolone resistance. In addition, one <i>Pseudomonas aeruginosa</i> isolate showed exceptional resistance to all β-lactams including carbapenems, aminoglycosides, and a new quinolone, showing a multidrug-resistant <i>P. aeruginosa</i> phenotype and remarkable biofilm formation. WGS of this isolate indicated that it belongs to ST235 and carries type I integron with multiple resistance genes, metallo-β-lactamase <i>bla</i>_{IMP-1}, aminoglycoside-resistance genes <i>aac(6')-Iae</i> and <i>aadA1</i>, and sulfamethoxazole-resistance gene <i>sull</i>.</p>			

Genome sequences comparison showed this isolate had a close clonal origin with the epidemic ST235 MDRP prevalent in Hiroshima region since 2005. One *Acinetobacter ursingii* isolate conferred extensive resistance to various β -lactams due to multiple acquired resistance genes, some of which were identified with the ones carried by the isolates reported from the Netherland, for examples, *bla*_{CARB-2}, *aac(6')Ib-cr*, *mph(E)*, *msr(E)*, *sull*, and *tet(39)*. On the other hand, she detected 6 *A. baumannii* isolates presented identical molecular characteristics and revealed more biofilm production than the others, strongly suggesting their clonal lineage. WGS data revealed these isolates belonged to the same sequence type, ST130. It was an interesting finding that besides the detection of ARB carrying multiple AMR genes, there was the presence of isolates with low minimum inhibitory concentration (MIC) based on biochemical tests while still growing on screening media plates with high biofilm-formation ability. This inconsistency reflects the difference in the AMR phenotype between a planktonic lifestyle of bacteria in MIC biochemical tests and biofilm lifestyle on agar plates, where bacteria are encased in an extracellular matrix that provides them tolerance and resistance mechanisms to combat antimicrobial challenges. Despite their low MICs by Vitek-2 or the lack of AMR genes, the growth of bacteria in the presence of an antimicrobial agent in the screening media may suggest their growability inside the human body with the presence of that antimicrobial agent. From the viewpoint of hospital infection control, the mobile AMR genes or plasmids are significant concerns due to their horizontal gene transfer from organism to organism within the health-care setting. However, from the viewpoint of clinical implications, both the AMR gene-carriers and biofilm-producers are threatening for their resistance phenotype inside the human body, either through the enzyme-mediated mechanism or the biofilm-based mechanism or through a combination of both. Strong biofilm-producing *A. baumannii* clone from this LTCF were regarded as a potential risk even though they lacked AMR genes.

Furthermore, she also collected the demographic data and clinical characterisations and analysed the risk factors. Two risk factors, strokes (cerebral infarction or cerebral haemorrhage) and percutaneous endoscopic gastrostomy tubes (PEG tubes) were significantly associated with the existence of ARB in the oral cavity. Patients underwent strokes were usually associated with disability, requiring substantial assistance of care-givers, for examples, dressing, toileting, eating, and other daily activities, hence facilitated the propagation of pathogenic microorganisms between patient-care givers and care givers-patient. In patients who were fed with PEG tubes, pathogenic colonisation inside the oral cavity might be promoted by a reduction in mastication activity and salivary secretion.

Base on these results, this dissertation greatly contributes to elucidate the prevalence and characteristics of drug-resistance bacteria in the oral cavity of the elderly in LTCF, as well as emphasizes the importance of professional oral care methods, such as brushing teeth, swabbing the mucosa, cleaning dentures, using mouthwash, having dental check-ups by professional dentists, improving the staff practices for oral care, and promoting oral hygiene, in reducing the risk of oral colonization of ARB, hence reducing the risk of aspiration pneumonia outcome in the elderly. Besides, this study also implies that health care workers involved in oral care should be aware of the existence of such ARB and pay attention to infection control to diminish the dissemination of ARB or the mobile resistance elements in LTCFs. Also, with the rapid ageing of the Japanese society, this study prompts the need for an expanded surveillance of ARB colonisation in a large-scale long-term care system to comprehend the general situation and comparative evaluation.

Therefore, all the committee members admitted that this dissertation is of sufficient value to confer the Doctor of Philosophy to Le Nguyen Tra Mi.