

論文内容要旨

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、リスク因子及び分子疫学の検討)
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Background: Residents of long-term care facilities (LTCFs) have been widely considered as reservoirs of antimicrobial-resistant bacteria (ARB), yet the oral colonization of those pathogens in this population, which potentially leads to aspiration pneumonia, remains unclear. This study aimed to investigate the prevalence, phenotypic characteristics, and molecular epidemiology of antimicrobial-resistant (AMR) Gram-negative bacteria in the oral cavity of LTCF residents, and to analyse the risk factors for such carriers.

Methods: Ninety-eight residents of an LTCF in Hiroshima City, aged between 55 and 101 years, were included in this study. 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 results were interpreted according to the Clinical Laboratory Standards Institute M100-S25. Biofilm formation was tested *in vitro*. Identification of epidemic clones was pre-determined by PCR; AMR genes, sequence types (ST), and whole-genome comparisons were conducted using whole-genome sequences (WGS). Demographic data and clinical characterisations were collected and risk factors analysed.

Results and Discussion: Fifty-four isolates from 37 patients (38%) were detected as ARB using screening agar plates and primarily belonged to three main genera: *Acinetobacter* spp. (35%), *Enterobacteriaceae* spp. (22%), and *Pseudomonas* spp. (19%). Among these 54 ARBs, 46 isolates from 32 patients (33%) were confirmed as having reduced susceptibility to one or more tested antimicrobial agents, 29 isolates from 23 patients (23%) were confirmed as cephalosporin-resistant ARB, and 13 isolates from 13 patients (13%) were confirmed as carbapenem-resistant ARB by Vitek-2.

All *Escherichia coli* isolates were resistant to various β -lactams and ciprofloxacin. WGS indicated these isolates belonged to ST131, serotype O25:H4, *fimH30*, and carried multiple plasmid-mediated AMR genes. In terms of β -lactam resistance genes, 3 *E. coli* isolates were found to carry *bla*_{CTX-M-27}, while 1 other *E. coli* isolate carried *bla*_{CTX-M-14} and *bla*_{TEM-1B}. Besides, these isolates also carried mutations in DNA sequences of the chromosomal quinolone resistance-determining regions of *gyrA* and *parC*, resulting in fluoroquinolone resistance.

One *Pseudomonas aeruginosa* isolate showed exceptional resistance to all β -lactams including carbapenems, aminoglycosides, and a new quinolone, showing a multidrug-resistant *P. aeruginosa* (MDRP) 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 *bla*_{IMP-1}, aminoglycoside-resistance genes *aac(6')-Iae* and *aadA1*, and sulfamethoxazole-resistance gene *sul1*. 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 displayed extensive resistance to various β -lactams due to multiple acquired resistance genes. *A. ursingii* is an uncommon opportunistic pathogen; sporadic cases involving serious bloodstream infections, in patients that are either immunocompromised or immunocompetent, have been reported. Our *A. ursingii* isolate carried some AMR genes identified with the ones carried by the isolates reported from the Netherland, for example, *bla*_{CARB-2}, *aac(6')Ib-cr*, *mph(E)*, *msr(E)*, *sul1*, and *tet(39)*.

Six *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. Besides the detection of ARB carrying multiple AMR genes, the presence of isolates with low minimum inhibitory concentration (MIC) based on biochemical tests and no acquired AMR genes while still growing on screening media plates, and high biofilm-formation ability, was also a noteworthy finding. This inconsistency, in fact, 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. The growth of bacteria in the presence of an antimicrobial agent, despite their low MICs by Vitek-2 or the lack of AMR genes, 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 given 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 important 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* isolates from this LTCF should be regarded as a potential risk even though it lacks AMR genes.

Two risk factors, strokes (cerebral infarction or cerebral haemorrhage) (OR 3.46, 95 % CI 1.38-8.70, $p = 0.007$) and percutaneous endoscopic gastrostomy tubes (PEG tubes) (Fisher's exact test, $p = 0.002$), indicated the existence of ARB in the oral cavity. Stroke was significantly associated with disability in the elderly, requiring substantial assistance from care-givers with regard to dressing, toileting, eating, and other daily activities. The greater the advanced disability patients have, the more assistance and interaction with care-workers and medical-device operators are required, consequently facilitating the propagation of such microorganisms. 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.

Conclusions: Our study detected a high prevalence of AMR Gram-negative bacteria, ESBL-producing and carbapenem-resistant pathogens relevant to aspiration pneumonia, which carried the resistance genes on mobile elements such as plasmids or integrons or in the chromosome and/or are strong biofilm producers, in the oral cavity of LTCF residents. 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, should be thoroughly considered and implemented. Health care workers involved in oral care should perceive the existence of such ARB and acquire the skills for infection control and prevention to diminish the dissemination of ARB or the mobile resistance elements in LTCFs. Last but not least, with the rapid ageing of the Japanese society, surveillance initiatives and regional and national projects for infection control should not put aside the significant role of LTCFs or nursing homes in the healthcare network.