The study of drug-resistant bacterial load and microbial safety of Bangladeshi ready-to-eat food

[課程博士・論文博士共通]

博士学位論文要約
Summary

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論文題目 Title	The study of drug-resistant bacterial load and microbial safety of Bangladeshi ready-to-eat food (バングラデシュの非加熱喫食調理済み (RTE) 食品の薬剤耐性菌汚染と微生物学的安全性に関する研究)		

Microbiological quality in ready-to-eat (RTE) foods is well studied but until now, many developing countries like Bangladesh, have not been made to determine multi-drug resistance (MDR) levels and associated resistance mechanisms in these foods. MDR bacteria are extensively distributed in the environment as an intermediary to transfer antibiotic resistance (AMR) genes between the environment and humans by direct contact or indirectly through the consumption of contaminated foods and establish themselves in the intestine of immunocompromised people thereby may affect the efficacy of antibiotics. As a result, opportunistic infections caused by drug-resistant organisms are difficult to treat and sometimes result in the death of patients. Bangladesh is facing problems associated with MDR bacteria health-related complications. To stop/minimize the pandemic of antibiotic resistance there is an urgent need to identify MDR bacteria in RTE foods to ensure safety and to effectively combat the spread of AMR in food. This study addresses the presence of MDR bacteria from 149 RTE foods belonging to 11 food categories sold at retail in Dhaka, Chittagong, and Cox'sbazar.

In Chapter 2, to understand the bacterial and fungal microbiota of traditional fermented milk products (three dahi, chanar-misti, paneer, borhani, sandesh, and baghabari ghee) made in Bangladeshi urban areas and Tokyo, Japan were analysed. The microbial properties were evaluated by classical culture-dependent and culture-independent methods with amplicon sequencing of the 16S rDNA (V4) and internal transcribed spacer (ITS) region with the MiSeq system. The viable lactic acid bacteria count was 6 and 8 log colony-forming units (CFU)/g. The yeast count was approximately 3 to 8 log CFU/g. The indigenous and/or contaminating microorganisms varied depending on the sample. For example, a high abundance of about 2 to 7 log CFU/g of gram-negative bacteria was detected in culture methods. Pyrosequencing results revealed the existence of gram-negative bacteria *Acinetobacter*, *Enterobacteriaceae*, *Aeromonadaceae*, and *Moraxellaceae*. However, the results of chapter-2, suggest that some of these traditional foods are undesirable RTE foods due to the high number of gram-negative bacteria. To verify the results, more RTE food products were analysed in chapter-3.

In Chapter 3, a total of 221 isolates were obtained from 141 Bangladeshi RTE food products, such as fried, non-fried and cooked foods; egg-, milk-, cereal-, and cream-based foods; pickles/achar; fruit; and RTE leaves, through culture on trypticase soy (for aerobic plate count), mannitol salt (for *Staphylococcaceae*), DHL (for *Enterobacteriaceae*), and NGKG (for *Bacillus cereus*- like bacteria) agar plates. The aerobic plate counts ranged from undetectable to 8.5 log CFU/g. After enrichment with peptone water, from 55%, 78%, and 55% of samples, contaminated bacteria were detected on DHL, mannitol salt, and NGKG agar plates, respectively. Twenty out of 111 isolates on DHL agar, 9 of 79 isolates on MSA agar, and 17 of 32 isolates on NGKG agar clearly showed resistance against three or more drugs (no clear zone around the paper disk). Through 16S ribosomal DNA sequencing analysis, six selected isolates from

DHL agar were identified as *Klebsiella pneumoniae* subsp. *pneumoniae*, *Citrobacter freundii*, *Enterobacter cloacae*, *Serratia marcescens*, *Pseudomonas nitroreducens*, *Pseudomonas plecoglossicida*, and two isolates from MSA were identified as *Staphylococcus gallinarum*, *Staphylococcus sciuri*, and one selected isolate from NGKG agar was identified as *Bacillus cereus*-like bacteria. The results suggest that antibiotic-resistant bacteria are ubiquitous in RTE foods distributed in Bangladesh. The growth/survival of highly MDR *Klebsiella pneumoniae* and *Citrobacter freundii* in six popular RTE foods including chotpoti, fuska (spicy snack of potato and chickpeas), cream bun (cookies), basil seed juice, egg chop, and betel leaves were conducted. During storage at 30 °C for 24 h, both strains showed greater growth in four samples approximately ≥ 8 log CFU/g and continued to grow up to 72 h. In contrast, the viable counts in basil seed juice and betel leaf samples were below the detectable limit (< 3 log CFU/g) after 48 h of storage. Interpretation of results shows that MDR bacteria can survive for long periods and may vary depending on the RTE food compositions. This chapter provides information on the type of MDR microorganisms to be suspected and needs whole-genome sequencing (WGS) for antibiotic resistance mechanisms and genes for toxicity.

In Chapter 4, the antibiotic resistance profile, pathogenesis potential, and virulence of *Klebsiella pneumoniae* (RTE-E3) and *Citrobacter freundii* (RTE-E5) were investigated. RTE-E3 and RTE-E5 were found to show resistance to 9 and 12 respectably, out of 20 antimicrobials tested. WGS analysis revealed that compared with type strain *Klebsiella pneumoniae* ATCC13883, 17 AMR genes including *blaSHV11*, *blaSHV81*, *blaSHV110*, *ampH*, *oqxA*, *oqxB*, *qnrS1*, *aph (3'')-Ib*, *aadA2*, *mph(A)*, *dfrA12*, *sul1*, *sul2*, *qacE*, *tetA, and fosA* were found in RTE-E3. A total of 17 antimicrobial resistance genes including *blaTEM-1B*, *blaDHA-1*, *blaCMY-129*, *blaCMY-77*, *qnrB4*, *qnrB10*, *qnrB17*, *qnrS1*, *aac(6')-Ib-cr*, *Mrx*, *mph (A)*, *mph (E)*, *msr (E)*, *dfrA14*, *qacE*, *tetA*, and *sul1* were detected in RTE-E5 as compared with type strain *Citrobacter freundii* ATCC8090. These genes encode resistance to extended-spectrum *β*-lactamase L-encoding genes including carbapenemase, quinolones, third-generation cephalosporin, sulfonamide, tetracycline, aminoglycoside, and fosfomycin respectively. Further, the presence of 16 antibiotic efflux pump-encoding resistance genes, including *AcrAB-TolC*, *AcrAD-TolC*, *AcrEF-TolC*, *AcrZ*, *EmrAB-TolC*, *EmrD*, *MacA*, *MacB*, *MdfA/Cmr*, *MdtABC-TolC*, *MdtL*, *MdtM*, *QacE*, *SugE*, *Tet(A), and TolC/OpmH* were detected in both strains. Moreover, MDR gene cluster- *mar(ABC)*, major facilitator superfamily- (*KpnG* and *KpnH*), and porin encoding- (*OmpK35* and *OmpK36*) genes were detected. Virulence analysis showed that the RTE-E3 strain carried virulence factor encoding genes such as *iutA*, *terC*, *ompR*, *cspC*, *mrkD*, *aroA*, *entB*, *fepC*, *fimH*, *ybtS*, *and MsgA* related to biofilm formation, motility, and iron uptake. RTE-E5 strain carried virulence genes such as *cspC*, *trxA*, *rpoS*, *ompR*, *gtrB*, *csgE*, *fimH*. *fliC*, *fliG*, *narG*, *entB*, *and fepC* related to endotoxin, fimbriae, biofilm formation, motility, and iron uptake.

The current study indicates the poor microbiological quality of several RTE food products prepared by vendors and sold in open markets or supermarkets in Bangladesh. The study demonstrated that the potential of MDR to survive in long display times (6-8hrs) at room temperature is possible and may also become the reason for MDR spread. The results suggest that bacterial pathogens such as *K. pneumoniae* and *C. freundii* with MDR and virulence phenotypes may be present in RTE food in Bangladesh. This study suggests that food safety training and application of the critical control points to ensure the quality and safety of RTE foods is highly needed. However, the policies and regulations that support the proper use of antibiotics as human medication, growth promoters of fish and animal farming, and crop culture are essential for effective interventions to contain the development and spread of AMR. At the same time implementation of effective policies and regulations to minimize the widespread availability of antimicrobials without prescription and self-medication is an urgent need to address the AMR challenge.