Antimicrobial resistance phenotype and distribution of RND multidrug efflux systems in Acinetobacter baumannii animal clinical isolates

S. Pagdepanichkit1,2, R Chuanchuen1

1Research Unit in Microbial Food Safety and Antimicrobial Resistance, 2Department of Veterinary Public Health, Faculty of Veterinary Sciences, Chulalongkorn University

*corresponding author: rchuanchuen@yahoo.com

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Introduction
Acinetobacter baumannii is one of the most important opportunistic bacteria, which is known as not only a major problem of community-acquired infection in humans but also a causative agent for nosocomial infection in animals (3). Multidrug resistant (MDR) A. baumannii has been increasingly reported in dogs, cats, horses and livestock worldwide (5). Multidrug efflux systems is one of the most common resistance mechanisms in A. baumannii, of which the resistance-nodulation-cell division (RND) family efflux systems are most frequently found in this organism (6). Up to date, 4 RND efflux systems have been identified in A. baumannii including AdeABC, AdeFGH, AdeJK and AdeDE (1,4). However, data of their distribution and antimicrobial resistance phenotype is still limited in A. baumannii animal clinical isolates.

Materials and Methods
Sample collection and isolation: the number of isolates were collected from all 210 samples. The samples were isolated from nasal swab obtained from animal carcasses (dogs, cats, birds, rabbits and exotic pets) that submitted for necropsy at Pathology Unit, Faculty of Veterinary Science, Chulalongkorn University during 2013 to 2014. Typical red colony on the selective medium, CHROMagar® Acinetobacter medium, which referred to Acinetobacter spp. were confirmed as A. baumannii species by using Amplified Ribosomal DNA Restriction Analysis (ARDRA) method (2).

Determination of antimicrobial susceptibility: The Minimal Inhibitory Concentrations (MICs) were performed using 2-fold agar dilution method according to Clinical and Laboratory Standard Institute guideline (CLSI). Fifteen clinically important antimicrobials were used in this study including amikacin (AMK), aztreonam (ATM), carbencillin (CAR), ceftazidime (CAZ), chloramphenicol (CHL), ciprofloxacin (CIP), erythromycin (ERY), gentamicin (GEN), kanamycin (KAN), neomycin (NEO), piperacillin (PIP), spectinomycin (SPE), streptomycin (STR), tetracycline (TET), trimethoprim (TMP).

Determination of distribution of RND multidrug efflux systems: Expression of RND multidrug efflux pump genes including adeB, adeG, adeJ and adeE were determined by using Reverse Transcription Polymerase Chain Reaction (RT-PCR). Specificity of the assay was confirmed by sequencing of PCR amplicons.

Results and Discussion
Of all 210 samples, 30 samples were positive to A. baumannii (14.29%). The A. baumannii animal isolates were most frequently found in dogs (17/30, 56.67%), cats (8/30, 26.67%), rabbits (4/30, 13.33%) and pigs (1/30, 3.33%). Our results showed that most of the isolates were obtained from companion animals. However, one pig sample was positive to A. baumannii. From these observations, A. baumannii was not circulated only in companion animals but also disseminated in livestock.

Twenty of 30 isolates were multidrug resistance (70%) and all the isolates were resistant to chloramphenicol (100%). High resistance rates were observed for trimethoprim (97%), erythromycin (93%) and spectinomycin (83%). The predominant resistance pattern was CHL-ERY-SPE-TMP (13%), followed by AMK-ATM-CAR-CAZ-CHL-CIP-ERY-GEN-KAN-NEO-PIP-SPE-STR-TET-TMP (6.7%). The expression of adeB, adeG and adeJ was detected in 53%, 90% and 100%, respectively. None of the isolates were expressed adeE. The expression patterns of the RND efflux pump were divided into 3 patterns including AdeB-AdeG-AdeJ (53%), AdeG-AdeJ (37%) and AdeJ (10%). These results suggested that there is the wide spread of multidrug resistance among A. baumannii animal clinical isolates. The wide distribution of RND efflux systems among these isolates was also demonstrated in this study.

The findings of the present study suggested that the expression of RND efflux systems could play an important role in the dissemination of multidrug resistance phenotype among A. baumannii animal clinical isolates. Further study is required to explore the exact contribution of these efflux systems to antimicrobial resistance in the A. baumannii clinical isolates.

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