Journal of Microbiology, Immunology and Infection (2017) 50, 653–661

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jmii.com

ORIGINAL ARTICLE

The plasmid-mediated fosfomycin resistance determinants and synergy of fosfomycin and meropenem in carbapenem-resistant *Klebsiella pneumoniae* isolates in Taiwan

Sung-Pin Tseng [a,b], Sheng-Fan Wang [a], Ling Ma [c], Ting-Yin Wang [a], Tsung-Ying Yang [a], L. Kristopher Siu [c], Yin-Ching Chuang [d,e], Pei-Shan Lee [f], Jann-Tay Wang [g], Tsu-Lan Wu [h], Jung-Chung Lin [i], Po-Liang Lu [f,j,k,*]

[a] *Department of Medical Laboratory Science and Biotechnology, College of Health Sciences, Kaohsiung Medical University, Kaohsiung, Taiwan*
[b] *Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Kaohsiung, Taiwan*
[c] *National Institutes of Infectious Diseases and Vaccinology, National Health Research Institutes, Miaoli, Taiwan*
[d] *Department of Internal Medicine and Medical Research, Chi Mei Medical Center, Tainan, Taiwan*
[e] *Department of Internal Medicine, Chi Mei Medical Center, Liouying, Tainan, Taiwan*
[f] *Department of Laboratory Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan*
[g] *Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan*
[h] *Department of Clinical Pathology, Linkou Chang Gung Memorial Hospital, Taoyuan, Taiwan*
[i] *Division of Infectious Diseases and Tropical Medicine, Department of Internal Medicine, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan*
[j] *College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan*
[k] *Center for Infectious Disease and Cancer Research, Kaohsiung Medical University, Kaohsiung, Taiwan*

Received 7 September 2016; received in revised form 13 February 2017; accepted 13 March 2017
Available online 28 June 2017

KEYWORDS
Fosfomycin;
Carbapenem resistance;

Abstract *Background:* Epidemiology of fosfomycin susceptibility and the plasmid-mediated fosfomycinase genes of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) isolates in Taiwan remain unclear.
Methods: 642 CRKP clinical isolates were collected from a nation-wide surveillance study (16 hospitals) in Taiwan in 2012–2013. Antimicrobial susceptibilities were determined. PFGE and

* Corresponding author. Department of Internal Medicine, Kaohsiung Medical University Hospital, 100, Shih-Chuan 1st Road, Kaohsiung, Taiwan. Fax: +886 7 3228547.
E-mail address: d830166@gmail.com (P.-L. Lu).

http://dx.doi.org/10.1016/j.jmii.2017.03.003

fosA3;
foskp96;
bla$_{KPC-2}$

MLST determined the clonal relatedness. Carbapenemases and fosfomycinases genes were detected by PCR, and their flanking regions were determined by PCR and sequencing. Synergistic activity of meropenem with fosfomycin was examined by the checkerboard method.
Results: In total, 36.4% (234/642) of CRKP isolates in Taiwan were resistant to fosfomycin. Among 234 fosfomycin-resistant CRKP isolates, PFGE analysis revealed 81 pulsotypes. Pulsotype XXIII (n = 63) was predominant and belonged to ST11. 71 had carbapnemases (65 *bla*$_{KPC-2}$-positive, 1 *bla*$_{VIM-1}$-positive and 5 *bla*$_{IMP-8}$-positive) and 62 had fosfomycinases (35 *fosA3*-positive and 27 *foskp96*-positive). Only 18.5% (5/27) of *foskp96*-positive isolates carried *foskp96* and *bla*$_{KPC-2}$, while 71.4% (25/35) of *fosA3*-positive isolates contained *fosA3* and *bla*$_{KPC-2}$. There were five types of flanking sequences for *fosA3*, and 85.7% (30/35) of *fosA3* genes were flanked by IS*26*, suggesting possible horizontal gene transfer. Synergistic effect of fosfomycin and meropenem was observed in all 25 randomly selected pulsotype XXIII strains (100%; 25/25), even those containing fosfomycinase (48%, 12/25) or carbapnemase (96%, 24/25).
Conclusions: A clone (pulsotype XXIII, ST11) has been found to be prevailing among fosfomycin-resistant CRKP in Taiwan. According to the *in vitro* data, the combination of fosfomycin and meropenem is a potentially alternative choice.

Introduction

Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is highly resistant to many antibiotics and is an emerging problem worldwide.[1–3] Fosfomycin is a treatment option,[4,5] a cell-wall active antimicrobial agent effective against gram-positive and gram-negative microorganisms.[5,6] Resistance mechanisms to fosfomycin include modification of the antibiotic target (MurA), functionless transporters (GlpT and UhpT), gene regulation (such as *uhpA*, *cyaA*, and *ptsI*), and fosfomycinases (*fosA3*, *fosC*, and *foskp96*).[7] Fosfomycinases can be disseminated by conjugative plasmid or transposon elements, and have led to serious problems in Japan, Korea and Hong Kong.[8–10] However, the epidemiology of fosfomycin susceptibility and the role of plasmid-mediated fosfomycinases in CRKP in Taiwan are unclear. The aim of this study was to survey fosfomycin susceptibility and plasmid-mediated fosfomycin resistance determinants in CRKP isolates from Taiwan. Furthermore, combinations of fosfomycin and meropenem were tested using clonally spreading fosfomycin-resistant CRKP strains.

Materials and methods

Bacterial strains

A total of 642 CRKP clinical isolates were collected from a nation-wide surveillance study from 16 hospitals in Taiwan in 2012 and 2013. The CRKP isolate definition was resistance to either imipenem or meropenem, according to CLSI 2012 guidelines.

Antimicrobial susceptibility testing

Antimicrobial susceptibility was tested by the agar dilution method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI).[11] The following antibiotics were tested: amikacin, aztreonam, ceftazidime, ciprofloxacin, cefepime, gentamicin, sulfamethoxazole/trimethoprim, meropenem, imipenem, doripenem, and fosfomycin. All antibiotic were obtained from Sigma–Aldrich.

Genotyping by PFGE and MLST

Epidemiological investigation was performed by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). The PFGE typing of XbaI-digested DNA (New England BioLabs, Ipswich, MA) was performed as previously described.[12] Restriction fragments ranging from 50 to 500 kb were separated using a CHEF Mapper apparatus (Bio-Rad) for 20 h at 200 V and 14 °C. The gels were then stained with ethidium bromide and photographed under UV light. Dice similarity indices were employed to construct a dendrogram of pulsotype relationships through the unweighted pair group method using arithmetic averages (UPGMA) with BioNumerics software version 6.5 (Applied Maths). Pulsotypes were assigned to the same clusters if they exhibited 80% similarity in the dendrogram. The MLST scheme of *K. pneumoniae* uses internal fragments from seven housekeeping genes: *rpoB* (beta-subunit of RNA polymerase), *gapA* (glyceraldehyde 3-phosphate dehydrogenase), *mdh* (malate dehydrogenase), *pgi* (phosphoglucose isomerase), *phoE* (phosphorine E), *infB* (translation initiation factor 2), and *tonB* (periplasmic energy transducer). Primers were derived from the *K. pneumoniae* MLST database (http://bigsdb.web.pasteur.fr/klebsiella/primers_used.html). PCR amplification and sequencing were performed following the protocols suggested on this website.

Detection of carbapenemases and fosfomycinases

Plasmid DNA was extracted using the QIAGEN Plasmid Mini Kit (Valencia, CA, USA). For the detection of carbapenemases