# 行政院國家科學委員會專題研究計畫　成果報告

## 以表現基因庫系統性選殖台灣克雷伯氏肺炎桿菌對乙內醯胺類藥物的抗藥性基因

計畫類別：個別型計畫
計畫編號：NSC93-2314-B-002-071-
執行期間：93 年 08 月 01 日至 94 年 07 月 31 日
執行單位：國立臺灣大學醫學院內科

計畫主持人：方啟泰

計畫參與人員：王錦堂（指導教授）賴碩彥（研究助理）湯淑瑛（研究生）林稚容（研究生）

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# 行政院國家科學委員會專題研究計畫成果報告

## 以表現基因庫系統性選殖台灣克雷伯氏肺炎桿菌對乙內醯胺類藥物的抗藥性基因

## Systemic Cloning of Beta-lactams Resistance Genes in *Klebsiella pneumoniae* Using Expression DNA Library

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### 一、中文摘要

克雷伯氏肺炎桿菌是一種天生對 ampicllin 及其他乙內醯胺類（beta-lactam）抗生素具有抗藥性的臨床上重要的致病菌。經由建構克雷伯氏肺炎桿菌菌株的 λ-ZAP II phage 表現基因庫，並且篩選具有 beta-lactam 抗藥性的轉型株，我們發現 SHV-1a（14/24）及 SHV-1（7/24）是使台灣的社區性克雷伯氏肺炎桿菌具有抗 ampicllin 能力的主要基因。侵襲性菌株（*n*=12）與非侵襲性菌株（*n*=12）在 beta-lactamase 基因型上並無顯著差別。某些台灣的社區性菌株也帶有 SHV-27（1/24）、SHV-41（1/24）及 TEM-116（24/24）。此三者先前被報稱為屬於 ESBL，但經轉殖實驗證明並非如此。在造成院內感染的 ESBL 菌株方面，我們發現 TEM-1b（23/48）及 SHV-12（16/48）是在台灣最常見的 ESBL 基因型，其次則為 CTX-M3（10/48）。但 48 株 ESBL 菌株中有 9 株的基因型不屬於已知的 SHV、TEM 及 CTX-M。

**關鍵詞**： 克雷伯氏肺炎桿菌、抗藥性、乙內醯胺類藥物

### Abstract

*Klebsiella pneumoniae* is an important pathogen inherently resists to ampicllin and sometimes other beta-lactams. Through constructing λ-ZAP II phage expression libraries of *K. pneumoniae* genome and screening beta-lactam-resistant transformants, we found SHV-1a ( 14/24 ) andSHV-1 ( 7/24 ) are the most common genetic basis of ampicillin resistance in community-acquired *K. pneumoniae* strains in Taiwan. There is no significant difference in the beta-lactamase genotypes between strains causing primary liver abscess （*n*=12） and strains did not cause primary liver abscess （*n*=12）. SHV-27 （1/24）, SHV-41 （1/24） and TEM-116( 24/24 ), which have been reported as encoding extended-spectrum beta-lactamase (ESBL) but not confirmed in the present study, were also identified in some community-acquired strains. In contrast, TEM-1b ( 23/48 ) and SHV-12 ( 16/48 ) are the predominant ESBL genotypes in nosocomial strains, followed by CTX-M3 （10/48）. Nine ESBL strains did not harbor known SHV, TEM or CTX-M genotypes.

Keywords: Drug Resistance, Beta-lactamases, *Klebsiella pneumoniae*

### 二、緣由與目的

*Klebsiella pneumoniae* is an enteric gram-negative bacillus which causes various nosocomial infections and septic shock in debilitated or immunocompromised patients [1–2]. In the past 15 years, a new type of invasive *K. pneumoniae* disease has emerged in Taiwan that typically presents as community-acquired primary liver abscess (PLA) with sepsis and bacteremia. Metastatic meningitis or endophthalmitis complicated

the course in 10–12% of cases [3–5]. Our previous research on the minimum inhibitory concentrations of various antimicrobial agents to invasive *K. pneumoniae* strains showed that, unlike the western *K. pneumoniae* strains which often demonstrate in vitro resistance to the first generation cephalo- sporins, these invasive strains are resistant to ampicillin only, but remain susceptible to all cephalosporins [6]. Since there is a lack of systemic genomic research on this phenotype, it is still not known whether there is a difference in genotype of beta-lactam resist- ance between the invasive and non-invasive *K. pneumoniae* strains. Therefore, we tried to isolate gene(s) on chromosome which are responsible for beta-lactam resistance in *K. pneumoniae* by using λ-Zap II expression libraries. PCR amplification was also used to isolate beta-lactam resistance genes on plasmids.

## 三、結果與討論

Expression genomic libraries of repre-sentative invasive *K. pneumoniae* strain NTUH-K2044 and a western genomic strain MGH-78578 were constructed and were transformed into ampicillin-sensitive *E. coli* XLOLR strain. Twenty clones of trans-formants were randomly selected from LB agar supplemented with ampicillin 100 mcg/ml. Two beta-lactam resistance genes were isolated and DNA sequencing result revealed one is SHV-1a and another is TEM-1. NTUH-K2044 carries SHV-1a only, while MGH 78578 carried both SHV-1a and TEM-1. Knockout of SHV-1a resulted in only a 32-fold decrease (from 4096 to 128 mcg/ml) in MICs of ampicillin to the NTUH-K-2044 strains.

To detect beta-lactamase genotypes in other *K. pneumoniae* strains on either chromosome or plasmids, PCR amplification was performed with following primers: CTGAATCATTATGCGTCCGG and CACCACCATCATTACCGAC for SHV-1 group; CCGACTATTTGCAA CAGTGC and GTTGCATCTATCTG GATGCC for SHV-5a group; CGCT CATGAGACAATAACCC and CAG TGAGGCACCTATCTC for TEM group; CGCTTTGCGATGTGCAG and ACCGCGATATCGTTGGT for CTX-M group; ATGAATGTCATTAT AAAAG and TTGGGCTTAGGGC AG for PER group. SHV-1a（14/24）andSHV-1（7/24） were found to be the most common genetic basis of ampicillin resistance in community-acquired *K. pneumoniae* strains in Taiwan. There is no significant difference in the beta-lactamase genotypes between strains causing primary liver abscess （*n*=12） and strains did not cause primary liver abscess （*n*=12）. SHV-27（1/24）, SHV-41（1/24） and TEM-116（24/24）were also identified in some community-acquired strains.

SHV-27 [7] and TEM-116 [8] were both previously reported as ESBL, however these 2 genes were detected in the non-ESBL producing strains in our study. Especially, TEM-116 was found in all of 24 non-ESBL producing strains. Therefore, we cloned these two genes into a pBK-CMV plasmid and then transformed into an *E. coli* DH10B strain. The *E. coli* DH10B strain was converted into ampicillin-resistant by transformation of SHV-27 and TEM-116 containing plasmid, but not produced ESBL. *E. coli* DH10B transformed with cloned SHV-5a produced ESBL, while cloned TEM-1 conferred ampicillin resistance but not ESBL producing phenotype. SHV-41 was also found in the ESBL-producing strain previously but not yet proven as ESBL [9]. However, SHV-41 was detected in our non-ESBL-producing isolate and trans-formation of SHV-41 containing plasmid also converted *E. coli* DH10B strain into ampicillin-resistant but not produced ESBL. We conclude that SHV-27, SHV-41 and TEM-116 are not ESBLs.

Unlike community-acquired strains, TEM-1b（23/48） and SHV-12（16/48） are the predominant genotypes in nosocomial ESBL strains, followed by CTX-M3（10/48）. Nine ESBL strains did not harbor known SHV, TEM or CTX-M genotypes.

We have identified three specific

genome regions in PLA strains, therefore, the genomic heterogeneity might also associated with antibiotic resistance pattern [10–12]. However, all PLA strains and noninvasive strains were ampicillin resistant, cefotaxime susceptible and none was ESBL-producing. Therefore, ESBL genotype is not associated with PLA.

As shown by previous studies [13–15], SHV-1a and SHV-1 were detected in most non-ESBL producing *K. pneumoniae* strains and SHV-12 (SHV-5a), TEM-1b and CTX-M3 were detected in most ESBL-producing isolates. TEM-116 was found in all of the community-acquired *K. pneumoniae* strains but in none of the 7 nosocomial ESBL isolates. The TEM-116 that has been identified in Korea recently was firstly reported in *K. pneumoniae* strains of Taiwan.

In our study, SHV-27 and TEM-116 were detected in non-ESBL-producing isolates, especially TEM-116 was found in all of the 24 community-acquired non-ESBL-producing strains. These two beta-lactamases were all identified as ESBLs previously because they were found in ESBL-producing isolates. SHV-41 was found in ESBL isolates before, however, its role on ESBL was not defined. By transformation of these 3 beta-lactamase genes into non-ESBL-producing *E. coli* DH10B strain, they did not produce ESBL phenotype. However, they conferred ampicillin resistance and protein expressions were further confirmed. Therefore, they are not real ESBL genes. Because no knock-out/complementation or transformation studies were done to confirm the ESBL gene function in the previous reports, therefore, there might be other genes responsible for ESBL producing in their strains.

The genetic basis of the beta-lactam resistance in the nine ESBL strains which did not harbor known SHV, TEM or CTX-M genotypes requires further study.

## 四、計畫成果自評

研究內容與原計畫相符程度：良好
達成預期目標情況：良好
研究成果的學術或應用價值：佳
是否適合在學術期刊發表：是 [16, 17]

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