行政院國家科學委員會補助專題研究計畫成果報告

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※　　　　利用表現基因庫以選殖克雷伯氏桿菌　　　　※
※　　　　　抗 Chlorhexidine 相關基因　　　　　　※
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計畫類別：■個別型計畫　　□整合型計畫

計畫編號：NSC－90－2320－B－002－151－

執行期間：民國九十年八月一日至民國九十一年七月三十一日

計畫主持人：　方啟泰

協同主持人：王錦堂教授、張上淳教授

計畫參與人員：陳豪川(研究生)、莊依萍(研究生)

本成果報告包括以下應繳交之附件：

□赴國外出差或研習心得報告一份

□赴大陸地區出差或研習心得報告一份

□出席國際學術會議心得報告及發表之論文各一份

□國際合作研究計畫國外研究報告書一份

執行單位：國立台灣大學醫學院內科

中　華　民　國　九　十　一　年　十　月　十　五　日

行政院國家科學委員會專題研究計畫成果報告

利用表現基因庫以選殖克雷伯氏桿菌抗 Chlorhexidine 相關基因

Molecular Cloning of Chlorhexidine Resistance Genes in *Klebsiellapneumoniae* Using Expression DNA Library

計畫編號：NSC 90-2320-B-002-151
執行期限：90 年 8 月 1 日至 91 年 7 月 31 日
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一、中文摘要

Chlorhexidine 是一種陽離子消毒抗菌劑，廣泛用於院內感染管制洗手消毒。我們建構對 chlorhexidine 具強抗藥性的克雷伯氏肺炎桿菌菌株的 ë-ZAP II 表現基因庫，並且篩選具有 chlorhexidine 抗藥性的轉型株。我們發現所有具有 chlorhexidine 抗藥性的轉型株均帶有一個含 903 核甘酸的基因，命名為 *cepA*。生物資訊學分析顯示 CepA 為一陽離子排出幫浦。在大腸桿菌 XLOLR 株表現 *cepA* 得到一 32.8-kDa 的蛋白質。將帶有 *cepA* 的質體送入大腸桿菌及克雷伯氏肺炎桿菌均造成 chlorhexidine 抗藥性增加。本研究結果顯示 *cepA* 與克雷伯氏肺炎桿菌的 chlorhexidine 抗藥性有關。CepA 可能使克雷伯氏肺炎桿菌能夠將 chlorhexidine 排出菌體外而產生抗藥性。

關鍵詞：六氯化物、抗藥性、克雷伯氏肺炎桿菌

Abstract

Chlorhexidine is a cationic biguanide antiseptic agent extensively used for handwashing in hospital infection control practice. We constructed a λ-Zap II expression library of a chlorhexidine-resistant *Klebsiella pneumoniae* strain and selected transformants with chlorhexidine resistance. A novel gene, *cepA*, with 903 nucleotides, was found to be present in all chlorhexidine-resistant clones. The predicted amino acid sequence was compatible with a cation efflux pump. Expression of *cepA* in *Escherichia coli* XLOLR yielded a 32.8-kDa protein. Retransformation using *cepA*-containing plasmid conferred chlorhexidine resistance to both *E. coli* XLOLR and a chlorhexidine-sensitive *K. pneumoniae* strain. We concluded that *cepA* is associated with chlorhexidine resistance in *K. pneumoniae*. The CepA protein may act as a cation efflux pump to export cations such as chlorhexidine out from bacterial cytoplasm.

Keywords: Chlorhexidine, Drug Resistance, *Klebsiella pneumoniae*

二、緣由與目的

Chlorhexidine is an extensively used handwashing antiseptics [1–3]. Some hospital-acquired gram-negative bacteria, including *Klebsiella*, *Serratia, Pseudomonas* etc., are resistant to chlorhexidine [4–8]. The mechanisms responsible for chlorhexidine resistance in gram-negative bacteria remain unclear. Therefore, we tried to isolate gene(s) responsible for chlorhexidine resistance in *K.*

pneumoniae by using λ-Zap II expression libraries.

三、結果與討論

Screening of phagemid expression library in *Escherichia coli* XLORL revealed 20 clones which grew on chlorhexidine 16 ì g/ml plates. DNA sequencing of the 20 clones revealed three different inserts. However, all clones contain a 903-nucleotide locus. Plasmid carrying this locus was retransformed into XLORL by 42°C heat shock. Retransformants again grew on plate containing 16 ì g/ml chlorhexidine.

Sequence of this open reading frame (ORF) showed a 90% similarity to *yiip*, a putative transporter in *E.coli* K12 [9]. The deduced amino acid sequence showed that it could be a cation efflux pump. Thus, this ORF was designed as *cepA* (GenBank accession number AB073019). Proteins display similarity to the deduced amino acid sequence of *cepA* included several putative permease proteins in *E. coli* and two putative transmembrane efflux proteins in *Salmonella typhi*. The adjacent ORF upstream and downstream to *cepA* had a nucleotide sequence 87% similar to *E. coli* transcription factor (*cpxR*) and 87% similar to *Enterobacter cloacae* phosphofructokinase (*pfkA*), respectively. Chromosome and plasmid were separated by pulsed field gel electrophoresis. Results of a nested PCR using *cepA*-specific primer pairs showed that *cepA* was present on the chromosome.

pBK-CMV plasmids carrying *cepA* were also transformed into NTUH-9770 by electroporation. Transformants had a four-fold increase in chlorhexidine MICs. Transformation with suicide vector *lacZ-cepA*-pUTKm1Ä*tnp*Ä*mini-Tn5* into ATCC 25922 yielded *cepA* single integration recombinants (confirmed by PCR with different alignments of primer pairs), which had a two-fold increase in chlorhexidine MICs.

No chloramphenicol-resistant transformants were obtained by transformation of linear DNA *cat*::*cepA* or intragenic fragment in a suicide vector. Transformations with suicide plasmid vector *cat*::*cepA*-pUTKm1 Ä*tnp*Ä*bla*Ä*mini-Tn5* yielded chloramphenicol-resistant clones. However, PCR amplification with PT-F1 and PT-R1 yielded both 1-kb and 1.8-kb products, corresponding to the size of wild type *cepA* and *cat::cepA*, respectively. This result suggested an integration, not a knock-out.

cepA was expressed in XLOLR transformants carrying *cepA*::pBK-CMV as previous described [10, 11]. A pBK-CMV without *cepA* was used as a control. SDS-PAGE with Coomassie Blue staining revealed a 33-kDa protein, the predicted size of CepA, in XLOLR carrying *cepA*::pBK-CMV but not in vector control.

Chlorhexidine is a cationic biguanide that kill bacteria by damaging membrane followed by intracellular coagulation [12]. Gram-negative bacteria are less susceptible to chlorhexidine than gram-positive bacteria [2, 3]. Impermeability of outer membrane to chlorhexidine has been implicated in *Pseudomonas*, *Proteus*, and *Providencia* (11, 18). Formation of biofilm prolongs survival of *Serratia* and *Burkholderia* on exposure to chlorhexidine [2, 3]. Chlorhexidine-degradating enzyme has been discovered in *Achromobacteria xylosoxidans* [13].

We demonstrated that transformation of *cepA*-containing phagemid into XLOLR resulted in siginificant elevations of chlorhexidine MICs. Single integration of *cepA* was sufficient to increase chlorhexidine MICs in *E. coli* by two-fold. Transformation of *cepA*-containing pBK-CMV into NTUH-9770 also resulted in a four-fold elevation of chlorhexidine MICs. These results suggested that *cepA* is associated with chlorhexidine resistance. Attempts using three different experiments failed to obtain knockout mutants. Therefore, *cepA* is probably essential. Our findings indicate that drug efflux might be an important mechanism of chlorhexidine resistance in *K. pneumoniae* and possibly in other related gram-negative bacteria. Similar mechanism is observed in staphylococci in which export proteins, encoded by *qacA/B,* are also responsible for chlorhexidine resistance [14, 15]. Hand-washing with chlorhexidine has failed to

control nosocomial spread of methicillin-resistant *S. aureus* with *qacA/B*-mediated resistance (MICs 2–4 ì g/ml) [16]. Because higher levels of MICs, chlorhexidine-resistant *K. pneumoniae* could be clinically more problematic than staphylococci.

In conclusion, *cepA* is associated with chlorhexidine resistance in *K. pneumoniae*. CepA protein may act as a cation efflux pump.

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：已發表 [17, 18]

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