北醫學報　第十一卷　1980年十二月
p.3-14

病原性大腸桿菌之研究
第V報　Spectinomycin 耐性莢膜多醣類產生變異株之特性

曾金章　曾美容　陳美玉*

大腸桿菌非病原性之NON-CPS株，以N-methyl-N′-nitro-N-nitroso-guanidine（NTG）處理之spectinomycin（SP）耐性菌株，根據其在PGS培養基及對抗生素之反應等得(1)SP^r-CPS-R變異株，它在PGS培養基產生粘稠菌落及對多種抗生素產生耐性；(2)另一群CPS株却是抗生素感受性之SP^r-CPS-S變異株；(3)非粘稠菌落及抗生素感受性之SP^r-NON-CPS變異株等三群。

SP^r-CPS-R變異株對各種抗生素之耐性限閥及交叉耐性率較SP^r-CPS-S及SP^r-NON-CPS變異株或原母株具明顯差異，而後兩者之反應則類似原母株，但SP^r-CPS-S變異株對抗生素之抵抗能力較SP^r-NON-CPS變異株及原母株強，菌株之藥物耐性受界面活性劑之影響。

SP^r-CPS-R變異株對多種碳水化合物獲得利用能力，但却消失了對adonitol的利用能力，及對天竺鼠紅血球有凝集反應。SP^r-變異株具較強之產毒性，尤其是SP^r-CPS-R變異株。

緒　論

大腸桿菌之莢膜多醣類（CPS）產生能力與其病原性、生化特性及抗生素耐性具很密切的關係[1-4]，以chloramphenicol（CM）及oxytetracycline（OTC）誘導非莢膜多醣類產生株（NON-CPS株）之CPS變異株都具類似之Patterns[4]。是否CPS的改變即意味著病原性、生化特性及抗生素反應等之改變仍未明瞭。

Spectinomycin（SP）是aminoglycoside類的抗生素，是細胞蛋白質生合成抑制劑[5-7]，它作用於ribosomes或ribosomal protein而導致細菌細胞膜機能或架構的改變，尤其是細菌表面構造之envelopes[8-15]，故本文選擇對抗生素感受性，非病原性之NON-CPS株，以NTG處理，並經SP選擇之。擬就此等變異株之特性提出報告，並探討莢膜產生能力與抗生素耐性之關係。

台北醫學院微生物學科　＊目前任職於本院分析化學科
民國六十九年九月受理

材 料 及 方 法

菌株[1]：得自臨床糞便來源株，包括E-2，3，6，26，27及28等6株，全部是對多種抗生素感受性，非病原性之NON-CPS株，保存于半固體之Nutrient broth (Difco)中，它們對SP之MIC是12.5—25.0μg/mℓ。

CPS變異株之分離[15-16]：以生理食塩水清洗18小時之Heart-infusion (HI；Difco)培養液3次後，添加Tris buffer (PH 6.3)泡製之NTG (N．B．Co) 50 μg/mℓ (細菌懸浮液濃度約等於MacFarland Nepherolometer Standards No.2) 處理30分鐘，再以生理食塩水清洗3次後置於含SP 100 μg/mℓ的HI琼脂平板培養基培養48小時，選擇在proteose - peptone No.3 glycerine salt (PGS) 培養基產生粘稠菌落之SP耐性變異株 (SP^r- CPS變異株)，並保存于HI半固體培養基，由于大部分變異株容易變爲NON-CPS之revertants，故本實驗以較安定之E-2變異株進行。

變異株之生化特性測定[3-4]，包括抗生素之感受性，碳水化合物之利用，抗原性及病原性等，並與SP^r-NON-CPS變異株及原母株比較之。

對各種抗生素抵抗能力之測定[3-4]：以生理食塩水各別清洗SP^r-CPS-S變異株，SP^r-NON-CPS變異株及原母株3次之18小時HI培養液0.1 ml (MacFarland Nepherolometer Standards No.3) 於5 ml含定量抗生素之生理食塩水中，包括gentamicin (GM, 先靈．80 μg/mℓ)，cefazolin (CEF，藤澤；100μg/mℓ)，kanamycin (KA，萬有．500μg/mℓ)，mitomycin-C (MMC，協和醱酵；50μg/mℓ)，streptomycin (SM,明治；250μg/mℓ)，oxytetracycline (OTC,輝瑞；1000μg/mℓ)，tetracycline (TC; 立達．1000μg/mℓ)，及carbenicillin (CARB，輝瑞；1000 μg/mℓ)，定時取一白金耳於10 mℓ HI broth，觀察其生長情形。

對界面活性劑的感受性及其處理菌株對抗生素感受性之測定：以Sodium lauryl sulfate (SLS;Wako)，Cetyltrimethyl ammonium bromide (CTMAB; Fluka)及Sodium deoxycholate (SOC;Difco)測定變異株對Surfactants之MIC，並以20 μg/mℓ的SLS及10 μg/mℓ的CTMAB處理，經生理食塩水清洗3次之18小時HI培養液 (6×10^9 或 3×10^{10} cells/mℓ)的SP^r-CPS-R，CM^r-CPS及OTC^r-CPS變異株[4] 10分鐘，並再經生理食塩水3次清洗後，取0.1 mℓ處理菌液于5 mℓ含各種不同濃度之Ampicillin (AP), KA及CARB之HI broth，觀察以界面活性劑處理前後菌株對抗生素MIC之變化，處理菌液分別計算其活菌數。

結 果

SP^r-CPS變異株之變異率 (表1)：E-2株之變異率最高在196SP^r-變異株中29株 (14.8%) 是CPS變異株，E-26株最低 (0.9%)，平均變異率是2.3%。SP^r-CPS變異株保存期間 (1-2代接種) 易恢復爲NON-CPS之revertants。Revertants對藥物之感受性除對SP外一如原母株。但E-2株之27株SP^r-CPS變異株則非常安定 (3年)。在31株E-2變異株中，根據其在PGS培養基之菌落形態及對抗生素之反應等分成三群(1)在27 SP^r-CPS變異株中20株是SP^r-CPS-R變異株，它們在PGS培養基產生粘稠菌落及對多種抗生素產生耐性，(2)另7株却對抗生素感受性故叫SP^r-CPS-S變異株(3) 4株非粘稠菌落及抗生素感受性之SP^r-NON-CPS變異株 (包括2株revertants)。

SP^r-CPS-R變異株對抗生素之感受性，(1)以HI琼脂平板連續稀釋法測定變異株之

Table 1. Mutation rates of the CPS mutants among the spectinomycin-resistant strains after treatment with NTG of wild type strains

Strains	Isolated No.	Mucoid mutants (%)	Backmutation (%)
E-2	196	29 (14.8)	2 (6.9)
E-3	244	4 (1.6)	2 (50.0)
E-6	487	6 (1.2)	4 (66.7)
E-26	217	2 (0.9)	1 (50.0)
E-27	179	3 (1.7)	2 (66.7)
E-28	277	3 (1.1)	3 (100.0)

Table 2 Susceptibility to various antibiotics of Escherichia coli mutants

Drug*	Geometric mean MIC (μg/ml)**			
	SP^r-CPS-R	SP^r-CPS-S	SP^r-NON-CPS	Parent+
CM	466.6	336.5	353.6	250.0
SP	500.0	234.6	281.2	12.5
EM	500.0	18.6	29.7	25.0
GAB	500.0	10.3	7.4	6.3
AP	500.0	3.5	3.7	6.3
CARB	500.0	3,8	5.3	3.2
NA	500.0	4.6	3.7	3.2
KA#	500.0	3.2	3.2	3.2
NEO	420.5	3.2	3.2	3.2
SM	271.3	3.2	3.2	3.2
MIM	190.0	3.2	3.2	3.2
RIF	26.8	37.2	29.7	12.5
MMC	6.9	6.9	5.3	3.2
CEF	20.3	3.2	3.2	3.2
COL	5.4	3.2	3.2	3.2
GM	3.2	3.2	3.2	3.2
Sum	185.4	6.5	6.5	5.1

Tested strains: SP^r-CPS-R: 20 strains, SP^r-CPS-S: 7 strains, and SP^r-NON-CPS: 4 strains (included 2 strains of CPS revertants).

*Chloramphenicol (CM), spectinomycin (SP), erythromycin (EM), aminosidine (GAB), ampicillin (AP), carbenicillin (CARB), nalidixic acid (NA), kanamycin (KA), neomycin (NEO), streptomycin (SM), minocycline (MIM), rifampicin (RIF), mitomycin-C (MMC), cefazolin (CEF), colistin-M (COL), and gentamicin (GM).

**For calculation of the geometric mean MIC, a value of 3.2 and 500.0 μg/ml was used in all cases where the MIC was $3.2 \geqslant$ and > 250.0 μg/ml.

+ Strains which developed mucoid (CPS) or nonmucoid (NON-CPS) colonies on proteose-peptone No. 3 glycerine salt agar. (1)

The results for oxytetracycline, methacycline, dememe methylchlortetracycline, chlortetracycline, tetracy cline and doxycycline were the same as that kanamycin.

Table 3. Comparison of geometric mean MICs of parent strain to spectinomycin resistance mutants

Drug*	MIC ratio of parent strain with		
	SP^r-CPS-R	SP^r-CPS-S	SP^r-NON-CPS
NEO	131.4	1.0	1.0
SM	84.8	1.0	1.0
CARB	79.4	1.2	1.7
GAB	79.4	1.6	1.2
NA	79.4	1.4	1.2
KA#	79.4	1.0	1.0
AP	79.4	−1.8	−1.7
MIM	59.4	1.0	1.0
SP	40.0	18.8	22.5
EM	20.0	−1.3	1.2
CEF	6.3	1.0	1.0
MMC	2.2	2.2	1.7
RIF	2.1	3.0	2.4
CM	1.9	1.3	1.4
COL	1.7	1.0	1.0
GM	1.0	1.0	1.0
Average	36.4	1.3	1.3

* See table 2 for definition of antibiotics abbreviation.

#The results for oxytetracycline, methacycline, deme methylchlortetracycline chlortetracycline, tetracycline and doxycycline were the same as that kanamycin.

MIC（表 2），除 GM， rifampicin（RIF）MMC及 colistin-M（COL）外，SP^r-CPS-R 變異株對抗生素之MIC 都有很明顯的提高，它對 22 種抗生素之平均 geometric mean MIC（185.4 μg/mℓ）不但較原母株（5.1 μg/mℓ）有明顯的差異，而且與 SP^r-CPS-S 及 SP^r-NON-CPS 變異株亦有相當明顯的提高（6.5μg/mℓ）。SP^r-CPS-R 變異株對 22 種抗生素之 geometric mean MIC較原母株相差 36.4 倍，較 SP^r-CPS-S及 SP^r-NON-CPS 變異株高 28.5 倍（表 3及表 4）。其中對KA，OTC，methacycline（MC），demethylchlortetracycline（DMCT），chlortetracycline（CT），TC，doxcycline（DOX），nalidixic acid（NA），NEO，CARB，AP，SM，GAB，minocycline（MIM）及 erythromycin（EM）等之 geometric mean MIC由16.8 倍到 156.3 倍以上之差異。(2)以對藥物之耐性及交叉耐性加以分析（表 5），20 SP^r-CPS-R 變異株具非常明顯的高抗生素交叉耐性率。其中 15 株（75.0 %）對 22 種抗生素之 19 種，4 株（20.0 %）對 20 種（12.9 %）及 1 株（5.0 %）對 21 種產生多劑交叉耐性。而 7 株 SP^r-CPS-S 及 4 株 SP^r-NON-CPS 變異株與原母株之抗生素耐性形態非常類似。

變異株之生化特性：(1) SP^r-CPS-R 變異株對碳水化合物之利用如表 6 所示，對 sucrose，rhamnose，ducitol 及 xylose 是 gain mutation，其他變異株則類似原母株。sucrose，dulcitol，xylose 及 adonitol 之利用能力和對抗生素之感受性或其他生化特性具有很密切的關連性。Adonitol 利用

Table 4. Comparison of geometric mean MICs of SP^r-CPS-R mutants to others

Drug*	MIC ratio of SP^r-CPS-R with		
	Parent	SP^r-NON-CPS	SP^r-CPS-S
KA#	156.3	156.3	156.3
NA	156.3	135.1	108.7
NEO	131.4	131.4	131.4
CARB	156.3	94.3	131.6
AP	79.4	135.1	142.9
SM	84.8	84.8	84.8
GAB	79.4	67.6	48.5
MIM	59.4	59.4	59.4
EM	20.0	16.8	26.9
SP	40.0	1.8	2.1
CEF	6.3	6.3	6.3
COL	1.7	1.7	1.7
CM	1.9	1.3	1.4
MMC	2.2	1.3	1.0
GM	1.0	1.0	1.0
RIF	2.1	−0.7	−0.9
Average	36.4	28.5	28.5

* See table 2 for definition of antibiotics abbreviation.
#The results for oxytetracycline, methacycline, deme methylchlortetracycline, chlortetracycline, tetracycline and doxycycline were the same as that kanamycin.

Table 5. Multiple drug resistance of Escherichia coli mutants

Drug combinations*	No. of positive strains			
	SP^r-CPS-R (20)	SP^r-CPS-S (7)	SP^r-NON-CPS (4)	Parent (1)
CM,SP,EM,RIF,GAB,AP,CARB,NEO,NA,SM,KA, OT,MC,DCT,CTC,TC,DOX,MIM,CEF,COL,&MMC	1 (5.0)#	0	0	0
CM,SP,EM,RIF,GAB,AP,CARB,NEO,NA,SM,KA, OT,MC,DCT,CTC,TC,DOX,MIM,CEF & COL	1 (5.0)	0	0	0
CM,SP,EM,RIF,GAB,AP,CARB,NEO,NA,SM,KA, OT,MC,DCT,CTC,TC,DOX,MIM,CEF & MMC	3 (15.0)	0	0	0
CM,SP,EM,RIF,GAB,AP,CARB,NEO,NA,SM,KA, OT,MC,DCT, CTC,TC,DOX,MIM & CEF	15 (75.0)	0	0	0
CM,SP,EM,RIF & MMC	0	0	1	0
CM,SP,EM,RIF & GAB	0	4	1	0
CM,SP,EM,& RIF	0	3	2	1

* See table 2 for definition of antibiotics abbreviation.
#Percentage.

Table 6. Carbohydrates ultilization of CPS mutants of Escherichia coli induced by NTG

Strains (tested number)	No. of positive strains (%)	Ultilization of													Antibiotics susceptibility**
		MAN#	GLU	LAC	ARA	SUC	SOR	TRE	RHA	DUL	RAF	SAL	XYL	INO	ADO	
SP^r-CPS-R (20)	6 (30.0)	AG*	AG	AG	AG	AG	AG	AG	A	AG	–	–	AG	–	–	R
	2 (10.0)	AG	AG	AG	AG	AG	AG	AG	AG	A	–	–	AG	–	–	
	12 (60.0)	AG	AG	AG	AG	AG	AG	AG	A	A	–	–	AG	–	–	
SP^r-CPS-S (7)	2	AG	AG	AG	AG	–	AG	AG	–	–	–	–	–	–	A	S
	3	AG	AG	AG	AG	–	A	A	–	–	–	–	–	–	A	
	2	AG	AG	A	A	–	AG	A	–	–	–	–	–	–	A	
SP^r-NON-CPS (4)	1	AG	AG	AG	A	–	AG	A	–	–	–	–	–	–	A	S
	1	AG	A	A	A	–	A	A	–	–	–	–	–	–	A	
	1	A	A	A	A	–	A	A	A	–	–	–	–	–	A	
	1	A	A	A	A	–	A	A	–	–	–	–	–	–	A	
Parent (1)	1	AG	A	A	AG	–	AG	A	–	–	–	–	–	–	A	S

Mannitol (MAN), glucose (GLU), lactose (LAC), arabinose (ARA), sucrose (SUC), sorbitol SOR), trehalose (TRE), rhamnose (RHA), dulcitol (DUL), raffinose (RAF), salicin (SAL), xylose (XYL), inositol (INO) and adonitol (ADO).

* A: acid, G: gas, –: no reaction; R: drug resistance, S: drug sensitive.

**The drugs tested were ampecillin, carbenicillin, streptomycin, neomycin, kanamycin, nalidixic acid, cefazolin, oxytetracycline, methacycline, demethychlortetracycline, chlortetracycline, tetracycline, doxycyline and minocycline.

Table 7. Enteroserological types of CPS mutants of Escherichia coli induced by NTG

Strains (Tested No.)	Sero-group (Difco)	No. of strains
SP^r-CPS-R (20)	D	20
SP^r-CPS-S (7)	C	5
	D	1
	untypable	1
SP^r-NON-CPS (4)	A	1
	B	2
	C	1
Parent (E–2)	B (086a:K61)	

能力消失之變異株都具較強之抗生素耐性。(2)以Difco腸血清型分型血清加以測定，其結果如表7所示，SP^r-CPS-R 變異株與原母株（086a；K61）之抗原性發生很大的變化，20 SP^r-CPS-R變異株屬于D群，7 SP^r-CPS-S變異株屬C群（5株），D群（1株）及非腸血清型（1株），2 revertants分屬A及B群，SP^r-NON-CPS 變異株分屬于B群及C群。(3)20 SP^r-CPS-R 變異株對天竺鼠紅血球產生凝集反應（heamagglutination），而其他變異株及原母株則呈陰性反應。

SP^r-CPS-R 變異株之病原性（表8），SP 耐性變異株較原母株具較高的產毒性，其中SP^r-CPS-R變異株之毒性最高，其次是SP^r-CPS-S變異株。

SP^r-CPS-S變異株對藥物之抗性並與藥物感受性之SP^r-NON-CPS變異株及原母株加以比較（表9），結果除MMC外，對其他抗生素之抗性都較SP^r-NON-CPS 變異株及原母株具相當明顯的差異，雖然SP^r-CPS-S 變異株在HI 瓊脂平板培養基對抗生素之MIC類似SP^r-NON-CPS 變異株及原母株，但却具

Table 8. Toxigenicity of CPS mutants of Escherichia coli induced by NTG

Strains		Mortality				
		< 4hr	8hr	12hr	24hr	Total
Parent (E—2)		—	—	—	—	0/8 (0)#
Mutants	SP^r-CPS-R	100.0	—	—	—	8/8 (100.0)
	SP^r-CPS-S	37.5	50.0	12.5	—	8/8 (100.0)
	SP^r-NON-CPS*	37.5	—	—	—	8/8 (37.5)
	SP^r-NON-CPS**	12.5	25.0	37.5	—	6/8 (75.0)

The denominator indicates the number of mice inoculated and the numerator is the number of mice died, figures in parenthesis show the percentage. Each of 8 mice was injected intraperitoneally with 0.5 ml of 12 hr stationary culture supernatants.
* Spectinomycin-resistant CPS revertant.
**Spectinomycin-resistant NON-CPS mutant.

Table 9. Resistance of wild type and NTG induced CPS mutants of Escherichia coli to antibiotics

Antibiotics (μg/ml)	MIC of tested strains (μg/ml)	Time of resistance (min)			
		Wild type	SP^r-CPS-S*	SP^r-NON-CPS**	SP^r-NON-CPS#
Gentamicin (80.0)	≤3.2	30	>300	<5	<5
Cefazolin (100.0)	≤3.2	30	>300	40	120
Kanamycin (500.0)	≤3.2	40	>300	<5	<5
Mitomycin-C (50.0)	≤3.2	25	30	<5	120
Streptomycin (250.0)	≤3.2	60	>300	<5	10
Oxytetracycline (1000.0)	≤3.2	<5	>300	<5	<5
Tetracycline (1000.0)	≤3.2	40	>300	<5	40
Carbenicillin (1000.0)	≤3.2	120	>300	<5	120

* This CPS mutant is drug sensitive to almost antibiotics.
** Spectinomycin-resistant CPS revertant was obtained from CPS mutants.
# Spectinomycin-resistant non-CPS mutant.

較長時間對藥物之耐性，故菌株之莢膜多醣類產生不但具較強之病原性而且會直接影響菌株對抗生素之反應。此種對抗生素之耐性是否以壁障（Barrier）阻斷抗生素之進入細胞因而提高其MIC 或抵抗性則尚未明瞭。

對界面活性劑之感受性（表10）SP^r-CPS-R變異株對CTMAB 之感受性較其他各群變異株高且及于SLS，是否與 cell envelope 所負電荷有關未明。

以20 μg/mℓ的 SLS及10 μg/mℓ的 CTMAB 處理 SP^r-CPS-R 變異株，CM^r-CPS 變異株及 OTC^r-CPS變異株後[4]，測

Table 10. Susceptibility of SP^r-CPS-R mutants of Escherichia coli to Sodium lauryl sulfate, Cetyltrimethyammonium bromide and Sodium desoxycholate on heart-infusion agar

Strains (No. of tested)	Geometric mean MIC (μg/ml)		
	CTMAB*	SLS	SDOC
SP^r-CPS-R (20)	0.2	12.5	>1,000
SP^r-CPS-S (7)	6.3	>1,000	>1,000
SP^r-NON-CPS (4)	1.9	>1,000	>1,000
Origin (1)	6.3	>1,000	>1,000

* CTMAB: Cetyltrimethylammonium bromide, SLS: Sodium lauryl sulfate, and SDOC: Sodium desoxycholate.

Table 11. Susceptibility to antibiotics of Escherichia coli mutants after surfactants treatment

Antibiotics	Treated with*	MIC (μg/ml)		
		SP^r-CPS-R	CM^r-CPS**	OTC^r-CPS**
Ampicillin	Control	500	>1000	⩾1000
	CTMAB	3.2 – 50	>1000	500
	SLS	⩾1000	>1000	⩾1000
Kanamycin	Control	50	50 – 100	500–>1000
	CTMAB	3.2 – 25	⩽1.8–6.3	100
	SLS	100 – 500	500	500
Carbenicillin	Control	>1000	>1000	>1000
	CTMAB	100 – 500	>1000	>1000
	SLS	>1000	>1000	>1000
CFU after surfactants treatment	Control	6.1×10^9	3.3×10^{10}	6.1×10^9
	CTMAB	3.8×10^7	3.0×10^7	4.3×10^5
	SLS	5.8×10^8	1.1×10^{10}	4.3×10^9

* 10 min after 20 μg/ml of Sodium lauryl sulfate(SLS) and 10 μg/ml of Cetyltrimethylammonium bromide(CTMAB) treatment, the treated cell suspension was washed three times with normal saline. 0.1 ml of these preparations was inoculation into certain concentration of various antibiotics contained heart-infusion broth. Results was observed for 48 hrs.

** Those mutants was obtained from chloramphenicol and oxytetracycline.(4)

定處理菌株對AP，KA及CARB等MIC 之變化（表11），雖然無法以此結果表示菌株之耐性是由莢膜多醣類當壁障所引起，但經界面活性劑處理之SP^r-CPS-R 變異株對抗生素之MIC都發生變化，尤其是CTMAB 處理株，當然和它活菌數可能有關。但SP^r-CPS-R變異株經LSL處理後，其MIC較未處理前高，這種結果顯示與界面活性劑即離子之種類有關。而且在經界面活性劑處理之CM^r-CPS及OTC^r-CPS變異株對抗生素MIC亦產生變化。

討　　論

在高濃度之 chloramphenicol 及 oxytetracycline 存在下，對藥物感受性及非病原性NON-CPS株大腸桿菌會突變產生CPS 變異株，而此種變異株不但提高對多種抗生素之MIC 及增加其交叉耐性率，且其生化特性、抗原性及產毒性與原母株亦具非常明顯的差異[4]。以NTG處理該菌株再經 spectiuomycin 選擇所產生的耐性CPS變異株中，20 SP^r-CPS-R變異株對 erythromycin 等15 種抗生素較其他變異株如 SP^r-CPS-S變異株，SP^r-NON-CPS變異株及原母株等之 geometric mean MIC 高出甚多，它們總平均相差約28.5倍到 36.4倍，而且對22種 抗生素中之 21種，20種及19種 產生交叉耐性，其耐性率分別是10.0％，15.0％及75.0％。而 SP^r-CPS-S及 SP^r-NON-CPS 變異株則類似原母株。由 SP^r-CPS-R變異株對抗生素之感受性、生化特性及病原性等加以衡量和 CM^r-及 OTC^r-CPS變異株頗爲相似[4]。

以 SP^r-CPS-R變異株，CM^r-CPS或 OTC^r-CPS變異株等來看，大腸桿菌在 PGS 培養基產生粘稠菌落之能力除與其對藥物感受性具密切關連外，也影響到菌株之生化特性、抗原性及病原性，而且都具類似之模式[4]，但NTG誘導變異株中同是粘稠菌落之 SP^r-CPS-S變異株，它們對抗生素之感受性，碳水化合物之利用及抗原性等之表現却類似 SP^r-NON-CPS 變異株及原母株，此等差異之表現是僅僅由於大腸桿菌所控制之莢膜多醣類產生之基因突變而使有關製造莢膜多醣類之酵素產生不同或不等量之莢膜多醣類[17-22]因而影響細菌包涵體之架構（configuration）導致滲透性之改變[15-23]，或同時引起多種的突變（random mutation）則尚未明瞭，而這些變異株却提供進一步研究的最佳素材。

CPS變異株對藥物耐性之獲得和細菌之藥物耐性因子之關係似乎不如細胞 envelopes所導致滲透性之改變[3-4,8-15,23-24]。Miyoshi 氏[15]發現大腸桿菌之sucrose-dependent-spectinomycin-resistant 變異株，對抗生素、染料及 detergent 等較原母株具很高的感受性（hypersensitivity）且具不正常之細胞形態。經 gel electrophoresis 及 polyacrylamide gel 分析其 cytoplasmic membrane 之蛋白質組成，在28個 protein band 中發現 Su^d-SPC^r 變異株常缺少 I-19，I-13及 I-24等 protein band[23]。

細菌對抗生素之耐性雖具多種方式，但 enzymatic inactivation 却是最被重視[25-29]，如 Shigella dysenteriae 所携帶之RTF更是家喻戶曉的事情[30]，但以細菌R-factors傳遞所引起之耐性來看，此種染色體外基因小體（exchromosomal genetics）所携帶之多劑耐性因子在 In vivo 並不像在 In vitro 那樣可以很容易在細菌間互相傳遞，故在醫院之病原菌其所具有之多劑耐性因子大部份來自醫院使用藥物之 selective pressure 的結果[31]由本研究之結果，藥物感受性之原母株在特殊情況下經抗生素等誘導，可以發現在 PGS 培養基產生粘稠菌落之 CPS 變異株，除如上述之種種異乎尋常之特性外，CPS 變異株之變異頻率不但很高（0.9％－14.8％）而且非常安定地繼續傳遞下去。故在細菌之藥物耐性中此種變異方式及耐性株之產生可能性很高應特予關注。

參 考 文 獻

1. TSENG CC: Studies on pathogenic Escherichia coli. I. Induction of capsular polysaccharide synthesis by sodium chloride in Escherichia coli and its pathogenicity to mice J Formosan Med Assoc 77; 328-335, 1978.
2. TSENG CC: Studies on pathogenic Escherichia coli. II. Pathogenicity of capsular polysaccharide-synthesizing Escherichia coli. J Formosan Med Assoc 77; 900-907, 1978.

3. TSENG CC, CHEN MY, LEE LW: Studies on pathogenic Escherichia coli. III. Multiple durg resistance of capsular polysaccharide-synthesizing Escherichia coli of clinical origins. J. Formosan Med Assoc 78; 7-16, 1979.
4. TSENG CC: Studies on pathogenic Escherichia coli. IV. Relation of capsular polysaccharide synthesis of antibiotic susceptibility, biochemical properties and pathogenicity. J Formosan Med Assoc 79; 500-510, 1980.
5. ANDERSON P: Sensitivity and resistance to spectinomycin in Escherichia coli. J Bacteriol 100; 939-947, 1969.
6. ANDERSOM P. DAVIES J, DAVIES BD: Effect of spectinomycin on polypeptide synthesis in extracts of Escherichia coli. J Mol Biol 29; 203-215, 1967.
7. DAVIES J, ANDERSON P, DAVIES BD: Inhibition of protein synthesis by spectinomycin. Science 149; 1096-1098, 1965.
8. BOLLEN A, DAVIES J, OZAKI M, MIZUSHIMA S: Ribosomal protein conferring sensitivity to the antibiotic spectinomycin in Escherichia coli. Science 165; 85-86, 1969.
9. DEKIO S, TAKATA R: Genetic studies of the ribosomal protein in Escherichia coli. II. Altered 30S ribosomal protein component specific to spectinomycin resistant mutants. Mol Gen Genet 105; 219-224, 1969.
10. HOLLAND JB, THRELFALL EJ, HOLLAND EM, DARBY V, SAMSON CR: Mutants of Escherichia coli with altered surface properties which are refractory to colicin E2, sensitive to ultraviolet light and which can also show recombination deficiency, abortive growth of bacteriophage λ and filament formation. J Gen Microbiol 62; 371-382, 1970.
11. NAKAMURA H: Genetic determination of resistance to acriflavine, phenethyl alcohol, and sodium dodecyl sulfate in Escherichia coli. J Bacteriol 96; 987-996, 1968.
12. NORMARK S: Mutation in Escherichia coli K-12 mediating spherelike envelopes and changed tolerance to ultraviolet irradiation and some antibiotics. J Bacteriol 98; 1274-1277, 1969.
13. SEIKIGUCHI M, IIDA S: Mutants of Escherichia coli permeable to actinomycin. Proc Natl Acad Sci U.S.A. 58; 2315-2320, 1967.
14. MANGIAROTTI G, APIRION D, SCHLESSINGER D: Selection of sucrose-dependent Escherichia coli to obtain envelope mutants and fragile cultures. Science 153; 892-894, 1966.
15. MIYOSHI Y, YAMAGATA H: Sucrose-dependent spectinomycin-resistant mutants of Escherichia coli. J Bacteriol 125; 142-148, 1976.
16. ADELBERG EA, MANDEL M, CHEN GCC: Optimal conditions for mutagensis by N-methyl-N'-nitro-N-nitrosoguanidine in Escherichia coli. Biochem Biophys Res Commun 18; 788-795, 1965.
17. KANG S, MARKOVITZ A: Induction of capsular polysaccharide synthesis by p-fluoro-phenylalanine in Escherichia coli wild type and strains with altered phenylalanyl soluble ribonucleic acid synthetase. J Bacteriol 93; 583-591, 1967.
18. BOURGEIS S, COHN M, DRGEL LE: Suppression of and complementation

among mutants of the regulatory gene of the lactose operon of Escherichia coli. J Mol Biol 14; 300-302, 1965.

19. CAPECCHI HR, GUSSIN GN: Suppression in vitro; identification of a serine-sRNA as a "nonsense" suppressor. Science 149; 417-422, 1965.
20. GAREN A, GAREN S: Genetic evidence on the nature of the repressor for alkaline phosphatase in Escherichia coli. J Mol Biol 6; 433-438, 1963.
21. GAREN A, SIDDIQUL O: Suppression of mutations in the alkaline phosphatase structural cistron of E. coli. Proc Natl Sci USA 48; 1121-1127, 1962.
22. MULLER-HILL BJ: Suppressible regulator constitutive mutants of the lactose system in Escherichia coli. J Mol Biol 15; 374-476, 1966.
23. MIZUNO T, YAMADA H, YAMAGATA H, MIZUSHIMA S: Coordinated alterations in ribosomes cytoplasmic membrane in sucrose-dependent, spectinomcyin-resistant mutants of Escherichia coli. J Bacteriol 125; 524-530, 1976.
24. TAMAKI S, SATO T, SUGINO Y, MATSUZAWA H, MATSUHASHI M: Studies on the mutants of E. coli sensitive to antibiotics. Progress in Antimicrobial and Anticancer Chemotherapy-Proceedings of the 6th International Congress of Chemotherapy, Volume 1; 405-409, 1970.
25. LEGOFFIC F, MARTEL A, WITCHITZ JL: 3-N enzymatic acetylation of gentamicin, tobramycin, and kanamycin by Escherichia coli carrying an R factor (errata). Antimicrob Agents Chemother 7; 386, 1975.
26. YAMAGUCHI M, KOSHI T, KOBAYASHI F, MITSUHASHI S: Phosphorylation of lividomycin by Escherichia coli carrying an R factor. Antimicrob Agents Chemother 2; 142-146, 1972.
27. PRICE KE, DEFURIA MD, PURSIANO TA: Amikacin, an aminoglycoside with marked activity against antibiotics resistant clinical isolates. J Inf Dis 134 (supplement); S 249-261, 1976.
28. BENVENSTR R, YAMADA T, DAVIES J: Enzymatic acetylation of streptomycin and spectinomycin by R-factor-resistant Escherichia coli. Infect Immun 1;109-119, 1970.
29. MITSUHASHI S, ROSIVAL L, KRCMERY V: Drug-inactivating enzymes and antibiotic resistance. Avicenum-Czechoslovak Medical Press. Prague Springer-Verlag Berlin Heidelberg New York, 2nd Internaitonal Symposium on Antibiotic Resistance, Castle of Smolenice, Czechoslovakia, 1974.
30. KITAMOTO O, FUKUMI H, ABE M, EZAKI T, MITSUHASHI S: Drug resistant Shigella in Japan. Progress in Antimicrobial and Anticancer Chemotherapy-Proceedings of the 6th International Congress of Chemotherapy, Volume II; 738-732, 1970.
31. KASS EH: Multiple drug resistane in the community outside the hospital. Progress in Antimicrobial and Anticancer Chemotherapy-Proceedings of the 6th Internaitonal Congress of Chemotherapy, Volume II; 746-748, 1970.

Studies on Pathogenic Escherichia coli (V). Preliminary Characterization of Spectinomycin-Resistant, Capsular Polysaccharide-Synthesizing Escherichia coli

Cheng-Chuang Tseng, Mei-Yung Tseng and Mei-Yi Chen

According to their colony types on the proteose-peptone No. 3 glycerine salt (PGS) medium and their susceptibility to antibiotics, the spectinomycin-resistant mutants were classified into the following three groups: Group 1: SP^r-CPS-R mutants. They grew mucoid colonies on the PGS medium and developed resistance to various antibiotics. Group 2: SP^r-CPS-S mutants. They grew mucoid colonies on the PGS medium but were sensitive to various antibiotics. Group 3: SP^r-NON-CPS mutants. They grew non-mucoid colonies on the PGS medium and were sensitive to various antibiotics.

The SP^r-CPS-R mutants showed significant different MIC resistance threshold and cross-resistance to various antibiotics as compared with SP^r-CPS-S mutants, SP^r-NON-CPS mutnats or the original parent strain. The SP^r-CPS-S mutants and SP^r-NON-CPS mutants reacted to antibiotics rather similarly as the original parent strain. However, the SP^r-CPS-S mutants showed longer survival time than SP^r-NON-CPS mutants and original parent strain against certain concentration of various antibiotics. The SP^r-CPS-R mutants were more susceptible to surfactants, their growth was inhibited much more than the other mutants, and their drug resistance was also affected by the surfactants.

The SP^r-CPS-R mutants gained ability of utilizing various carbohydrates, but could not utilize adonitol, and ability the guinea pig RBC heamagglutination. The spectinomycin-resistant mutants showed rather strong toxinogenicity, especially the SP^r-CPS-R mutants showed the strongest.

Department of Microbiology, Taipei Medical College
Received for Publication: September 1980.