

國科會九十年年度專題研究計畫成果報告

計畫名稱: 食用植物及其成份抑制抗藥性細菌生長之研究

編號: NSC 89-2320-B-040-060; NSC 90-2320-B-040-013 ✓

執行期限: 89-8-1 至 91-7-31

主持人: 殷梅津, 中山醫學大學營養學系

共同主持人: 曹世明, 中山醫學大學附設醫院感染科

一、中文摘要:

本研究旨在探討數種食用植物及其成份抑制五種院內抗藥性細菌(金黃色葡萄球菌、白色葡萄球菌、克雷白氏菌、綠膿桿菌、靜止桿菌)生長的能力及臨床應用的可能性。第一年度的研究重點有:(1) 檢測食用植物萃出液的 *in vitro* 抑菌能力;(2) 分析及比較此萃出液的抑菌成份。第二年度的研究重點有:(1) 抑菌成份的確認;(2) 特定抑菌成份與抗生素(如 ceftazidime、gentamicin、imipenem、meropenem) 的 combined 效果。

關鍵字: 食用植物、抗藥性細菌

Abstract:

The nosocomial infection caused by multidrug-resistant bacteria has been increased in past ten years in Taiwan. The most common isolates are *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*. The resistance of these pathogens against the common used antibiotics such as penicillin are higher than 70%.

Our past studies regarding the *in vitro* effects of plant foods for controlling nosocomial blood and wound fungal infection found that Allium plants, spicy plants and plants rich in phenolic compounds possessed marked antifungal activities. However, the information about the antibacterial effects of these foods and their components upon nosocomial bacterial infection control is lacked. The purposes of this project included: 1) to examine the inhibitory effect of plant foods and their compounds against these multidrug-resistant pathogens; 2) to study the interaction of antimicrobial compounds with antibiotics.

Keywords: multiple drug resistant bacteria, edible plant foods

二、緣由與目的:

在國內由於抗生素的大量濫用使得引發院內血流感染的抗藥性細菌及黴菌的種類及量快速增加。這其中最常見的抗藥性菌種有對 methicillin、penicillin 產生抗藥性的金黃色葡萄球菌(methicillin-resistant *Staphylococcus aureus*)、綠膿桿菌(*Pseudomonas aeruginosa*)及克雷白氏菌(*Klebsiella pneumoniae*)等。在國內的數個醫學教學中心, 這些菌對 penicillin、gentamicin、tetracyclines 等抗生素的抗藥性都已經超過 70%。本實驗室過去的研究發現蔥科植物、薑黃科植物及其 sulfides 成份都含有抑菌成份, 可以在 *in vitro* 的環境

下有效的抑制數種黴菌(念珠菌及麴菌)的生長。因此，本實驗室計畫以這些院內血流及傷口感染的抗藥性細菌及黴菌為對象，研究數種食用植物之特定成份的抑制抗藥性細菌的能力。

三、結果與討論：

本研究的結果顯示許多食用植物的萃出液及其內的特定成份，如 diallyl monosulfide, diallyl disulfide, diallyl trisulfide, diallyl tetrasulfide, curcumin 不但可以殺死抗藥性細菌，還可以與抗生素產生加成(additive)或加乘(synergistic)效果。如此不但可以抑制抗藥性細菌的生長及擴散，還可以減少藥物的使用劑量。另外，部份的人體試驗也已經進行，蔥科植物的水萃液在受試者身上也表現出顯著的抑菌效果。這些結果已投稿至 Journal of Medical Microbiology、Journal of Antimicrobial Chemotherapy 及 Journal of Food and Drug Analysis，這三篇都已經刊登。

四、計畫成果自評：

在預定的時間內達成了擬定的研究目的。並將具體成果發表三份 papers，都發表在 SCI 的期刊(附件 1-3)。因而自評：成果尚佳。

五、參考文獻：

1. 張智華、王復德 常見院內感染病原菌及其防治措施 感控雜誌 1997;7:194-203
2. Chen ML, Chang SC, Pan HJ, et al: Longitudinal analysis of methicillin-resistant *Staphylococcus aureus* isolates at a teaching hospital in Taiwan. J Formos Med Assoc 1999;98:426-432.
3. Hu BS, Lau YJ, Shi ZY, et al: Nosocomial *Klebsiella pneumoniae* bacteremia. 感控雜誌 1999;7:286-292.
4. 吳俊昇、張峰義 敗血症候群之新觀念 感控雜誌 1999;9:90-97.
5. Tsao SM, Yin MC. Enhanced inhibitory effect from interaction of curcumin with amphotericin B or fluconazole against *Candida* species. J Food Drug Analysis. 2000;8:208-212.
6. Tsao SM, Yin MC. In vitro antimicrobial activities of four diallyl sulfides naturally occurring in garlic and Chinese leek oils. J Medical Microbiol. 2001;50:646-649.
7. Tsao SM, Yin MC. In vitro activity of garlic oil and four diallyl sulfides against antibiotic-resistant *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. J Antimicrobial Chemother. 2001;47:665-670.

Inhibitory Effects of Aqueous Garlic Extract, Garlic Oil and Four Diallyl Sulphides against Four Enteric Pathogens

MEI-CHIN YIN^{1*}, HUI-CHING CHANG¹ AND SHYH-MING TSAO²

¹ Department of Nutritional Science, Chungshan Medical University, No. 110, Sec. 1, Chien-Kuo N. Rd., Taichung City, Taiwan, R.O.C.

² Department of Infection, Chungshan Hospital, Taichung City, Taiwan, R.O.C.

(Received: November 19, 2001; Accepted: January 22, 2002)

ABSTRACT

The inhibitory effects of aqueous garlic extract, garlic oil and four diallyl sulphides naturally occurring in this oil against *Escherichia coli*, *Enterobacter cloacae*, *Enterococcus faecalis*, and *Citrobacter freundii* (total 291 clinical isolates) were studied. The MIC values of four diallyl sulphides against the four enteric pathogens followed the order diallyl monosulphide > diallyl disulphide > diallyl trisulphide > diallyl tetrasulphide ($p < 0.05$). Most interactions of four antibiotics (meropenem, ceftazidime, imipenem and gentamicin) with diallyl polysulphide, determined as FIC index, showed synergistic or additive effects. Garlic oil at 2X MIC reduced original inoculum to $\leq 3 \log_{10}/\text{mL}$ within 8 hr; and 4X MIC reduced original inoculum to $< 2 \log_{10}/\text{mL}$ in all test enteric pathogens within 6 hr. The intake of aqueous garlic extract in humans provided the antibacterial activity in plasma, determined by inhibitory zone. These results suggested that aqueous garlic extract, garlic oil, and diallyl polysulphide may have potential for the prevention or control of infections caused by enteric pathogens.

Key words: garlic, diallyl polysulphides, enteric pathogens, antibacterial, human plasma

INTRODUCTION

Escherichia coli, *Enterobacter cloacae*, *Enterococcus faecalis*, and *Citrobacter freundii* are four common enteric pathogens responsible for nosocomial infections in Taiwan⁽¹⁻³⁾. These pathogens not only cause a variety of infections but also have developed resistance to many antibiotics such as ceftazidime and gentamicin^(4,5). The infections caused by these enteric pathogens further require expensive antibiotic treatments as well as increase the morbidity and mortality in patients. Thus, there is a need for the development of agents with marked antibacterial activity, greater stability and less toxicity.

Diallyl monosulphide (DAS), diallyl disulphide (DADS), diallyl trisulphide (DAT) and diallyl tetrasulphide (DATS) are four sulphide compounds found in onion, garlic and Chinese leek⁽⁶⁻⁸⁾. The antimicrobial activities of garlic oil and/or these sulphide compounds have been studied in our laboratory^(8,9) and others^(10,11). Consequently, it was reported that garlic oil was an effective antimicrobial agent; and the bactericidal activity of DAS, DADS, DAT and DATS against *Helicobacter pylori*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* was in proportion to the sulfur atoms present in these sulphides⁽⁸⁻¹¹⁾. In order to extend the understanding regarding the antimicrobial efficiency of garlic oil and its diallyl sulphides, our laboratory continues to examine and compare the inhibitory effects of garlic oil, and its four diallyl sulphides against four enteric pathogens, *E. coli*, *E. cloacae*, *E. faecalis* and *C. freundii*. The interactions of sulphide

agents with antibiotics were also investigated.

On the other hand, *in vitro* inhibitory effect of aqueous garlic extract (AGE) against several pathogens has been assayed^(12,13); however, it remains unknown that the intake of AGE enhances the antibacterial efficiency in humans. Therefore, the inhibitory effect of plasma collected from AGE-consuming humans was examined.

MATERIALS AND METHODS

I. Aqueous Garlic Extract (AGE) Preparation

Garlic bulbs (*Allium sativum* L.) were directly purchased from farms. One hundred g peeled edible portion was chopped and homogenized in 100 mL sterile distilled water in a Waring blender and followed by filtration through Whatman No. 1 filter paper. The filtrate was further sterilized by passing through a 22- μm -pore-size filter (Millipore, France). The filtrate was collected in a sterile vial and stored at 4°C until used.

II. Garlic Oil and Four Diallyl Sulphides Preparation

The method described in Tsao and Yin⁽⁹⁾ was used to prepare garlic oil. Diallyl monosulphide (purity 97%) and diallyl disulphide (purity 80%) were purchased from Aldrich Chemical Co. Diallyl disulphide was further purified by fractional distillation. Diallyl trisulphide and diallyl tetrasulphide were obtained by fractional distillation from crude diallyl disulphide. The purity of each diallyl sulphide was then examined by a HPLC method⁽¹⁴⁾, in which HPLC (Hitachi, Japan) was equipped with a Supelco (Bellefonte,

* Author for correspondence. Tel:04-24730022 ext. 1753; Fax:04-24739030; E-mail:mcyin@csmu.edu.tw

PA) LC18 column. These diallyl sulphides with purity greater than 95% were used in this study.

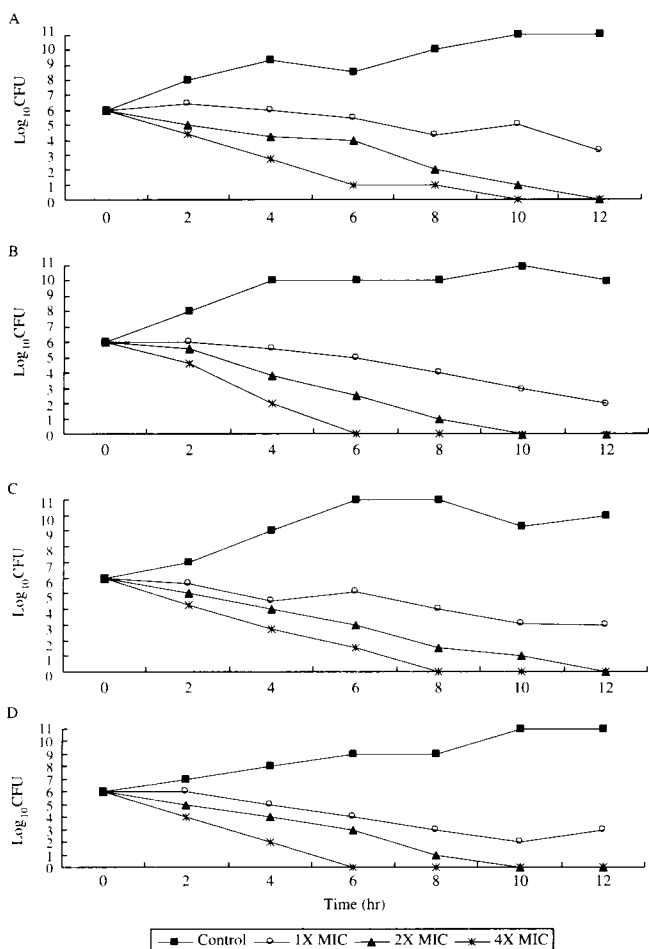


Figure 1. *In-vitro* time-kill of garlic oil at various concentrations against antibiotic-resistant *E. coli* (A, n=17), *E. cloacae* (B, n=11), *E. faecalis* (C, n=15) and *C. freundii* (D, n=12) within 12 hours.

III. Bacterial Strains and Medium

Escherichia coli, *Enterobacter cloacae*, *Enterococcus faecalis* and *Citrobacter freundii* were isolated from infected patients in Chungshan Hospital (Taichung City, Taiwan). The total numbers of clinical isolates of *E. coli*, *E. cloacae*, *E. faecalis* and *C. freundii* in this study were 75, 69, 81 and 66, respectively. All isolates were identified by Vitek (Vitek AMS; BioMerieux Vitek, Inc., Hazelwood, MO) and API 20E (API-BioMerieux, La Balme Les Grottes, France). Antibiotic-resistant profiles were determined by using discs with antibiotics (Difco, Detroit, MI) placed on the surface of nutrient agar (Difco, Detroit, MI) plates seeded with the test organism. Interpretation of resistance was based on the National Committee for Clinical Laboratory Standards (NCCLS) criteria⁽¹⁵⁾. The antibiotics used were meropenem, ceftazidime, imipenem and gentamicin, and the discs with 30 µg antibiotics were purchased from Sigma Chem. Co. (St. Louis) or Difco Laboratory (Detroit, MI). After identification, all cultures were routinely maintained on nutrient agar at 25°C until used. For each experiment, each culture was incubated overnight at 37°C, cells were then centrifuged, washed and resuspended in sterile peptone solution. Each culture was then serially diluted to yield a proper population for inoculation.

IV. Minimum Inhibitory Concentration Determination

AGE, garlic oil, DAS, DADS, DAT, and DATS were used for antibacterial tests. Both antibiotic-susceptible and antibiotic-resistant enteric pathogens were used to determine the MIC values of aqueous garlic extract, garlic oil and four diallyl sulphides. Microdilution MIC was determined with strains grown in cation-adjusted Mueller-Hinton broth (Difco, Detroit, MI) according to NCCLS guidelines⁽¹⁵⁾. The agent concentrations ranged from 128 to 0.125 µg/mL. All incubations were at 37°C, 24 hr.

Table 5. Inhibitory effect of human plasma against four enteric pathogens before and after consuming 250 mL aqueous garlic extract (AGE). Effect was determined by inhibitory zone (cm) and data were expressed as mean ± SD

	Before		After AGE		Consumption
		20 min	40 min	60 min	120 min
<i>E. coli</i>					
susceptible (23)	0.2±0.1	0.7±0.2	1.6±0.4	1.3±0.3	0.5±0.1
CZ ^a -resistant (21)	0	0.4±0.3	1.3±0.4	1.0±0.2	0.4±0.2
GE-resistant (18)	0	0.3±0.1	1.4±0.2	1.1±0.3	0.3±0.1
<i>E. cloacae</i>					
susceptible (19)	0.15±0.1	0.5±0.2	1.3±0.3	1.0±0.3	0.4±0.2
CZ-resistant (26)	0	0.3±0.1	0.9±0.2	0.7±0.3	0.2±0.2
GE-resistant (18)	0	0.3±0.2	1.2±0.3	0.9±0.2	0.4±0.1
<i>E. faecalis</i>					
susceptible (20)	0	0.6±0.3	1.4±0.2	1.2±0.2	0.4±0.2
CZ-resistant (26)	0	0.4±0.1	1.2±0.2	0.8±0.3	0.4±0.2
GE-resistant (17)	0	0.5±0.2	1.2±0.3	0.9±0.4	0.3±0.1
<i>C. freundii</i>					
susceptible (16)	0.15±0.1	0.4±0.2	1.3±0.3	1.0±0.1	0.5±0.2
CZ-resistant (24)	0	0.3±0.1	0.8±0.1	0.9±0.2	0.3±0.2
GE-resistant (23)	0	0.4±0.3	1.0±0.2	0.8±0.2	0.3±0.1

^aCZ, ceftazidime; GE, gentamicin.

V. Time Kill Study of Garlic Oil

Seventeen *E. coli*, 11 *E. cloacae*, 15 *E. faecalis* and 12 *C. freundii* clinical isolates were resistant to both ceftazidime and gentamicin in this study. *In-vitro* kill of garlic oil against these antibiotic-resistant strains was monitored in 10 mL volumes over 24 hr at 37°C after inoculation with cultures in cation-adjusted Mueller-Hinton broth without agitation. Aliquots of 100 μ L were cultured on solid medium at intervals for determination of cfu/mL and viable counts were read after 24 hr incubation. The limit of detection was 20 cfu/mL.

VI. Interaction of Diallyl Polysulphide with Antibiotics

The interactive relationship between each test diallyl sulphide agent and four antibiotics against both antibiotic-susceptible and antibiotic-resistant enteric bacteria were evaluated by the checkerboard method recommended by the NCCL⁽¹⁶⁾. One hundred μ L aliquots of each agent at 10X the targeted final concentration were used. Agent-agent interactions are classified as synergistic, additive or less-than-additive based on the fractional inhibitory concentration (FIC) index, which is the sum of FICs for each agent. The FIC of each agent is calculated as the MIC of this agent in combination divided by the MIC of this agent alone. Agent-agent interactions are considered synergistic if the FIC index is less than 1.0; additive, if the FIC is equal to 1.0; less-than-additive, if the FIC is higher than 1.0.

VII. Antibacterial Activity of Plasma

Twenty mL fresh whole blood was drawn from healthy 10 males and 9 females aged 20-23 after overnight fasting. All subjects then consumed 250 mL AGE (about 100 g edible garlic). Twenty mL blood was drawn respectively at 20, 40, 60 and 120 min after the AGE intake. Plasma was collected by centrifugation. Antibacterial activity of plasma was determined and compared by disc diffusion methods⁽¹⁵⁾. A blank disc was soaked in plasma for 30 min and then placed on the surface of nutrient agar plates previously seeded with 100 μ L test enteric bacteria. The inoculum concentration for each test bacteria was 106 cell/mL. The inhibitory zone was measured after 24 hr incubation at 37°C. The contents of DAS, DADS, DAT, and DATS in 250 mL AGE and plasma samples after AGE consumption were analyzed by a HPLC method⁽¹⁴⁾, and diallyl polysulphide was extracted with acetonitrile and quantified by a HPLC (Hitachi, Japan) equipped with a Supelco (Bellefonte, PA) LC18 column.

VIII. Statistical Analysis

The data for MIC, inhibitory zone and each point in time-kill curve were calculated as mean \pm SD. The interaction of each diallyl polysulphide with each antibiotic was analyzed from at least 12 different preparations ($n \geq 12$). Other data were evaluated by two-way analysis of variance.

RESULTS

In this study, there were no meropenem-, or imipenem-resistant enteric pathogens; and the resistance rates of these clinical isolates to ceftazidime and gentamicin were in the range of 15.6~29.7%. The MIC values of AGE, garlic oil and four diallyl sulphides against four test enteric pathogens are presented in Table 1 and followed the order AGE > DAS > DADS > DAT \geq garlic oil > DATS ($p < 0.05$). For each test organism, there was no significant difference in the MIC value of test agent between antibiotic-susceptible and antibiotic-resistant strains ($p > 0.05$).

The interactions of DADS, DAT or DATS with four antibiotics against both antibiotic-susceptible and antibiotic-resistant strains, determined as FIC index, are presented in Tables 2-4. Most interactions of diallyl polysulphides with four antibiotics against all test enteric pathogens were synergistic or additive because their FIC indexes were ≤ 1 . Only one combination, DADS plus ceftazidime against ceftazidime-resistant *C. freundii*, showed less-than-additive because its FIC index was > 1 (Table 2).

Consuming AGE enhanced the antibacterial effect of plasma, determined by inhibitory zone and showed in Table 5. The intake of AGE resulted in the plasma collected at 40 min and 60 min showed similar inhibitory zone ($p > 0.05$), which was significantly greater than the data obtained at 20 min and 120 min ($p < 0.05$). The contents of DAS, DADS, DAT and DATS in 250 mL AGE (100 g garlic bulb) were 9.52 ± 1.27 , 59.73 ± 4.41 , 92.34 ± 6.58 and 31.23 ± 2.64 mg, respectively. The limit of detection was 20 μ g/mL and the content of these sulphides in all plasma samples was too low to be detected.

The bactericidal effects of garlic oil determined by *in vitro* time-kill curves are displayed in Figure 1. The bactericidal effects of garlic oil increased significantly with increasing concentrations from 1X to 4X MIC. Garlic oil at 4X MIC effectively reduced the original inoculum to $< 2 \log_{10}$ /mL in all test organisms within 6 hr; and at 2X MIC reduced the original inoculum to $\leq 3 \log_{10}$ /mL in all test organisms within 8 hr.

DISCUSSION

The inhibitory effect of garlic oil and its four diallyl sulphides against methicillin-resistant *S. aureus*, fungi, *P. aeruginosa* and *K. pneumoniae* has been observed in our previous works^(8,9). The results of our present study extended the antimicrobial activities of these agents to four other medically important pathogens. When compared with our previous work⁽⁹⁾, garlic oil and its four diallyl sulphides, based on the relatively lower MIC values, showed a greater inhibitory effect against the four enteric pathogens than against *P. aeruginosa* and *K. pneumoniae*. This finding suggested these diallyl sulphides were more effective in controlling nosocomial infections caused by these enteric bacteria. A relationship between lower MIC values and greater number of sulfur atoms/molecule for diallyl sulphides has been observed in our previous works^(8,9) and others⁽¹⁰⁾. Our present study also

Table 1. Minimum inhibitory concentration ($\mu\text{g/mL}$) of garlic oil, aqueous garlic extract (AGE) and diallyl sulphides^a against antibiotic-susceptible and antibiotic-resistant enteric pathogens. Data were expressed as mean \pm SD

	Garlic oil	AGE	DAS	DADS	DAT	DATS
<i>E. coli</i>						
susceptible (23) ^c	8 \pm 2	44 \pm 4	32 \pm 4	24 \pm 2	12 \pm 2	4 \pm 2
CZ-resistant (21)	12 \pm 4	44 \pm 8	40 \pm 8	28 \pm 4	12 \pm 2	4 \pm 2
GE-resistant (18)	8 \pm 2	52 \pm 8	40 \pm 4	28 \pm 4	16 \pm 4	4 \pm 2
<i>E. cloacae</i>						
susceptible (19)	12 \pm 2	56 \pm 8	40 \pm 4	28 \pm 4	20 \pm 4	4 \pm 2
CZ-resistant (26)	12 \pm 4	64 \pm 8	48 \pm 8	32 \pm 4	24 \pm 2	8 \pm 2
GE-resistant (18)	12 \pm 4	64 \pm 4	40 \pm 8	36 \pm 4	20 \pm 4	8 \pm 2
<i>E. faecalis</i>						
susceptible (20)	12 \pm 2	64 \pm 8	40 \pm 8	28 \pm 8	16 \pm 4	8 \pm 4
CZ-resistant (26)	16 \pm 4	72 \pm 12	48 \pm 8	32 \pm 8	20 \pm 8	8 \pm 4
GE-resistant (17)	16 \pm 4	64 \pm 8	48 \pm 8	32 \pm 4	20 \pm 4	12 \pm 4
<i>C. freundii</i>						
susceptible (16)	8 \pm 4	64 \pm 8	56 \pm 8	40 \pm 8	20 \pm 4	8 \pm 2
CZ-resistant (24)	12 \pm 2	72 \pm 12	56 \pm 8	48 \pm 4	24 \pm 4	12 \pm 2
GE-resistant (23)	12 \pm 2	72 \pm 12	56 \pm 12	48 \pm 4	24 \pm 4	12 \pm 4

^a DAS, diallyl monosulphide; DADS, diallyl disulphide; DAT, diallyl trisulphide; DATS, diallyl tetrasulphide.

^b CZ, ceftazidime; GE, gentamicin.

^c number of strain.

Table 2. Interactions of diallyl disulphide (DADS) and antibiotics^a against both antibiotic-susceptible and antibiotic-resistant enteric pathogens. The interaction was determined as FIC index^b

	<i>E. coli</i>		<i>E. cloacae</i>		<i>E. faecalis</i>		<i>C. freundii</i>	
	susceptible	resistant	susceptible	resistant	susceptible	resistant	susceptible	resistant
DADS	0.5	0.5	0.5	0.5	0.375	0.625	0.625	0.625
CZ	0.25	0.375	0.25	0.5	0.5	0.375	0.25	0.5
FIC index	0.75	0.875	0.75	1.0	0.875	1.0	0.875	1.125
DADS	0.25	0.5	0.5	0.75	0.625	0.5	0.5	0.625
GE	0.5	0.375	0.25	0.25	0.25	0.5	0.375	0.375
FIC index	0.75	0.875	0.75	1.0	0.875	1.0	0.875	1.0
DADS	0.5	– ^c	0.375	–	0.5	–	0.5	–
IM	0.375	–	0.375	–	0.25	–	0.375	–
FIC index	0.875	–	0.75	–	0.75	–	0.875	–
DADS	0.5	–	0.5	–	0.5	–	0.75	–
ME	0.375	–	0.375	–	0.375	–	0.125	–
FIC index	0.875	–	0.875	–	0.875	–	0.875	–

^a CZ, ceftazidime; GE, gentamicin; IM, imipenem; ME, meropenem.

^b The interaction of DADS with antibiotics was evaluated by checkerboard method recommended by the NCCLs and expressed as the sum of fractional inhibitory concentration (FIC) index for each agent.

^c No IM- and ME-resistant enteric pathogens present in this study.

found a similar relationship in inhibiting these enteric bacteria because the MIC values were DATS < DAT < DADS < DAS. The finding once again agreed with those previous studies and supported that the number of sulfur atom/molecule and/or disulphide bond in these diallyl sulphides was an important factor in determining their antimicrobial activities. Garlic oil with effective bactericidal activity (Figure 1) may be considered as a functional food in clinical nutrition for prevention or control of infections.

The resistance of bacteria against commonly used antibiotics was due to the production of beta-lactamase, the increased pyrrolidonylarylamidase activity, aminoglycoside-modifying enzymes, and/or any alterations in different penicillin-binding proteins in the bacteria⁽¹⁷⁻¹⁹⁾. In this present study, 9 *E. coli* and 7 *E. faecalis* with beta-lactamase negative (determined by nitrocefin disc, data not shown) showed to be

both ceftazidime- and gentamicin-resistant. This finding once again demonstrated the complexity of antibiotics resistance. However, our previous works and the present study found garlic oil and its diallyl polysulfides were able to inhibit both antibiotic-susceptible and antibiotic-resistant strains, the inhibitory action mode of these agents was evidently different from that of known antibiotics. This advantage not only suggested the possibility of using these compounds independently as novel antibacterial agents but also partially explained the enhanced bactericidal efficiency observed in the combinations of diallyl polysulfides with antibiotics. In the present study, most combinations of diallyl polysulphide with these antibiotics were additive or synergistic; therefore, the application of these combinations may effectively inhibit these pathogens. Moreover, the used dosage of antibiotics in these combinations could be lower than when they are used

Table 3. Interactions of diallyl trisulphide (DAT) and antibiotics^a against both antibiotic-susceptible and antibiotic-resistant enteric pathogens. The interaction was determined as FIC index^b

	<i>E. coli</i>		<i>E. cloacae</i>		<i>E. faecalis</i>		<i>C. freundii</i>	
	susceptible	resistant	susceptible	resistant	susceptible	resistant	susceptible	resistant
DAT	0.375	0.375	0.375	0.5	0.25	0.375	0.5	0.5
CZ	0.25	0.375	0.25	0.375	0.5	0.375	0.375	0.5
FIC index	0.625	0.75	0.625	0.825	0.75	0.75	0.875	1.0
DAT	0.375	0.5	0.375	0.5	0.5	0.5	0.5	0.625
GE	0.25	0.25	0.25	0.25	0.25	0.325	0.375	0.25
FIC index	0.625	0.75	0.625	0.75	0.75	0.875	0.875	0.875
DAT	0.5	– ^c	0.5	–	0.625	–	0.375	–
IM	0.25	–	0.25	–	0.125	–	0.25	–
FIC index	0.75	–	0.75	–	0.75	–	0.625	–
DAT	0.375	–	0.5	–	0.5	–	0.375	–
ME	0.375	–	0.375	–	0.25	–	0.5	–
FIC index	0.75	–	0.875	–	0.75	–	0.875	–

^a CZ, ceftazidime; GE, gentamicin; IM, imipenem; ME, meropenem.

^b The interaction of DAT with antibiotics was evaluated by checkerboard method recommended by the NCCLs and expressed as the sum of fractional inhibitory concentration (FIC) index for each agent.

^c No IM- and ME-resistant enteric pathogens present in this study.

Table 4. Interactions of diallyl tetrasulphide (DATS) and antibiotics^a against both antibiotic-susceptible and antibiotic-resistant enteric pathogens. The interaction was determined as FIC index^b

	<i>E. coli</i>		<i>E. cloacae</i>		<i>E. faecalis</i>		<i>C. freundii</i>	
	susceptible	resistant	susceptible	resistant	susceptible	resistant	susceptible	resistant
DATS	0.25	0.375	0.375	0.5	0.25	0.375	0.5	0.5
CZ	0.25	0.25	0.25	0.25	0.375	0.375	0.25	0.375
FIC index	0.5	0.625	0.625	0.75	0.625	0.75	0.75	0.875
DATS	0.375	0.375	0.375	0.5	0.375	0.5	0.25	0.5
GE	0.125	0.25	0.125	0.125	0.375	0.25	0.5	0.25
FIC index	0.5	0.625	0.5	0.625	0.75	0.75	0.75	0.75
DATS	0.5	– ^c	0.5	–	0.625	–	0.375	–
IM	0.125	–	0.25	–	0.125	–	0.25	–
FIC index	0.625	–	0.75	–	0.75	–	0.625	–
DATS	0.375	–	0.5	–	0.5	–	0.375	–
ME	0.25	–	0.25	–	0.25	–	0.375	–
FIC index	0.625	–	0.75	–	0.75	–	0.75	–

^a CZ, ceftazidime; GE, gentamicin; IM, imipenem; ME, meropenem.

^b The interaction of DATS with antibiotics was evaluated by checkerboard method recommended by the NCCLs and expressed as the sum of fractional inhibitory concentration (FIC) index for each agent.

^c No IM- and ME-resistant enteric pathogens present in this study.

alone; and this may further reduce the occurrence of side effects caused by these antibiotics.

After AGE intake, only 3 subjects complained of stomach discomfort. The intake of AGE apparently modified the plasma composition, which resulted in the presence of antibacterial activity (Table 5). After checked with our available standards, no detectable DAS, DADS, DAT or DATS was found in the plasma. Therefore, the observed antibacterial activity in plasma might be due to the combined effect of these sulphides agents in trace amounts, or the presence of other antibacterial compounds such as allicin, ajoene in the AGE⁽²⁰⁾. Because of the lack of standards, the contents of allicin, ajoene and other components were not determined in the present study. Although the antibacterial components from AGE remained unknown, these results of Table 5 indicated that the antibacterial compounds from AGE could be absorbed and circulated into blood; and the maximum

inhibitory effect appeared at 40-60 min after intake. The four enteric pathogens tested in this study were responsible for bacteremia occurred in human^(3,21,22); thus, the regular intake of AGE, such as 250 mL every two hours, should be able to directly enhance the antibacterial capability of plasma; and further prevent deterioration of bacteremia. When compared with other agents tested in this study, AGE possess the advantages of easy preparation, low side effects and low cost. Therefore, AGE consumption may be an economic way for infected patients or hospital workers to control or prevent infections.

In conclusion, diallyl polysulphides, based on the marked individual antibacterial activities and combined effects with antibiotics, may be considered as new antibiotics. Aqueous garlic extract and garlic oil are two functional foods with antibacterial activity. The application of these two foods may benefit the prevention and treatment of clini-

cal bacterial infections.

ACKNOWLEDGEMENTS

This research was supported by a grant from the National Science Council, ROC [NSC 90-2320-B-040-013].

REFERENCES

- Chang, W. N., Lu, C. H., Huang, C. R. and Chuang, Y. C. 2000. Mixed infection in adult bacterial meningitis. *Infection* 28: 8-12.
- Jang, T. N., Kuo, B. I., Shen, S. H., Fung, C. P., Lee, S. H., Yang, T. L. and Huang, C. S. 1999. Nosocomial gram-negative bacteria in critically ill patients: epidemiologic characteristics and prognostic factors in 147 episodes. *J. Formos. Med. Assoc.* 98: 465-473.
- Shih, C. C., Chen, Y. C., Chang, S. C., Luh, K. T. and Hsieh, W. C. 1996. Bacteremia due to *Citrobacter* species: significance of primary intraabdominal infection. *Clin. Infect. Dis.* 23: 543-549.
- Ho, M., McDonald, L. C., Lauderdale, T. L., Yeh, L. L., Chen, P. C. and Shiau, Y. R. 1998. Surveillance of antibiotic resistance in Taiwan, 1998. *J. Microbiol. Immunol. Infect.* 32: 239-249.
- Chang, S. C., Hsieh, W. C. and Liu, C. 2000. High prevalence of antibiotic resistance of common pathogenic bacteria in Taiwan. *Diag. Microbiol. Infect. Dis.* 36: 107-112.
- Lawson, L. D., Wood, Z. Y. and Hughes, B. G. 1991. HPLC analysis of allicin and other thiosulfonates in garlic clove homogenates. *Planta Med.* 57: 263-270.
- Sparnins, V. L., Barany, G. and Wattenberg, L. W. 1998. Effects of organosulfur compounds from garlic and onions on benzo[a]pyrene-induced neoplasia and glutathione S-transferase activity in the mouse. *Carcinogenesis* 9: 131-134.
- Tsao, S. M. and Yin, M. C. 2001. *In vitro* antimicrobial activity of four diallyl sulphides occurring naturally in garlic and Chinese leek oils. *J. Med. Microbiol.* 50: 645-649.
- Tsao, S. M. and Yin, M. C. 2001. *In vitro* activity of garlic oil and four sulphides against antibiotic-resistant *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. *J. Antimicrob. Chemother.* 47: 665-670.
- O'Gara, E. A., Hill, D. J. and Maslin, D. J. 2000. Activities of garlic oil, garlic powder and their diallyl constituents against *Helicobacter pylori*. *Appl. Environ. Microbiol.* 66: 2269-2273.
- Ross, Z. M., O'Gara, E. A., Hill, D. J., Sleightholme, H. V. and Maslin, D. J. 2001. Antimicrobial properties of garlic oil against human enteric bacteria: evaluation of methodologies and comparisons with garlic oil sulfides and garlic powder. *Appl. Environ. Microbiol.* 67: 475-480.
- Pai, S. T. and Platt, M. W. 1995. Antifungal effects of *Allium sativum* (garlic) extract against the *Aspergillus* species involved in otomycosis. *Lett. Appl. Microbiol.* 20: 14-18.
- Sivam, G. P., Lampe, J. W., Ulness, B., Swanzy, S. R. and Potter, J. D. 1997. *Helicobacter pylori*-*in vitro* susceptibility to garlic (*Allium sativum*) extract. *Nutri. Cancer* 27: 118-121.
- Lawson, L. D., Wang, Z. J. and Hughes, B. G. 1991. Identification and HPLC quantification of the sulfides and dialk(en)yl thiosulfides in commercial garlic products. *Planta Med.* 57: 363-370.
- National Committee for Clinical Laboratory Standards. 1999. Performance Standards for Antimicrobial Susceptibility Testing. Ninth informational supplement M100-S9. National Committee for Clinical Laboratory Standards. Villanova, PA. USA.
- National Committee for Clinical Laboratory Standards. 1997. Performance Standards for Checkerboard Synergy Tests. National Committee for Clinical Laboratory Standards. Villanova, PA. USA.
- Cercenado, E., Vicente, M. F., Diaz, M. D., Sanchez-Carrillo, C. and Sanchez-Rubiales, M. 1996. Characterization of clinical isolates of beta-lactamase-negative, highly ampicillin-resistant *Enterococcus faecalis*. *Antimicrob. Agents Chemother.* 40: 2420-2422.
- Facklam, R., Pigott, N., Franklin, R. and Elliott, J. 1995. Evaluation of three disk tests for identification of enterococci, leuconostocs, and pediococci. *J. Clin. Microbiol.* 33: 885-887.
- Papaparaskevas, J., Vatopoulos, A., Tassios, P. T., Avlami, A., Legakis, N. J. and Kalapothaki, V. 2000. Diversity among high-level aminoglycoside-resistant enterococci. *J. Antimicrob. Chemother.* 45: 277-283.
- Agarwal, K. C. 1996. Therapeutic actions of garlic constituents. *Med. Res. Rev.* 16: 111-124.
- Wang, F. D., Liu, Y. M. and Liu, C. Y. 1998. Bacteremia in patients with hematologic malignancies. *J. Formos. Med. Assoc.* 97: 405-409.
- Christie, C., Hammond, J., Reising, S. and Evans-Patterson, J. 1994. Clinical and molecular epidemiology of enterococcal bacteremia in a pediatric teaching hospital. *J. Pediatr.* 125: 392-399.

大蒜水萃液、蒜油及其四種含硫成份抑制 四種腸內菌之功效

殷梅津^{1*} 張惠清¹ 曹世明²

1. 中山醫學大學 營養系
2. 中山醫學大學附設醫院 感染科
台中市建國北路一段110號

(收稿：November 19, 2001；接受：January 22, 2002)

摘 要

四種腸內菌 (*Escherichia coli*、*Enterobacter cloacae*、*Enterococcus faecalis* 及 *Citrobacter freundii*，共 291 隻臨床菌株) 被使用來探討蒜汁、蒜油及其四種含硫成份 (diallyl monosulphide、diallyl disulphide、diallyl trisulphide、diallyl tetrasulphide) 的抑菌能力，以及與抗生素 (meropenem、ceftazidime、imipenem 及 gentamicin) 共同使用時的抑菌效果。體外試驗發現，四種含硫成份對腸內菌的最低抑制濃度 (MIC) 為 DAS > DADS > DAT > DATS，且與抗生素共同使用時可表現出加成或加乘的效果；蒜油於 4 倍 MIC 時可在 6 小時內將菌數由 $6 \log_{10}/\text{mL}$ 降至 $2 \log_{10}/\text{mL}$ 以下。人體試驗發現，受試者飲用 250 mL 蒜汁後，因抑菌成份出現於血漿，而使得血漿在 1 小時內表現出抑菌效果 (以抑制圈檢測)。本研究結果支持蒜油的四種含硫成份具有成為新抗生素的潛力，不論單獨使用或與抗生素一起使用都將有助於治療腸內菌感染。而蒜油及蒜汁則可研發為機能性食品，應用於臨床營養以幫助住院患者預防或治療腸內菌感染。

關鍵詞：大蒜，含硫成份，腸內菌，抑菌能力，血漿