

**Antibacterial activity of oils of clove and garlic on bacterial isolates  
(special emphasis on coagulase- positive *Staphylococcus aureus* )  
from patients with septic wound (anapauk) from Traditional Medicine Hospital**

\*Lwin Lwin Cho, \*\*Mar Mar Nyein, \*Win Myint, \*Sein Win & \*\*\*May Kyi Aung

\*Traditional Medicine Research

\*\*Bacteriology Research Division (DMR,LM)

\*\*\*Microbiology Department, University of Medicine 2

Wound swab samples from 50 patients (age ranged from 13-69 years) attending Traditional Medicine Hospital from 25 January to 26 April 2005, were collected in Stuart's transport media. Isolation of bacterial pathogens was proceeded by standard methods, using Blood agar, Mannitol Salt agar, MacConkey agar, Nutrient agar and Ashdown agar. Further diagnosis was done by Gram staining, biochemical and serology reaction. Among them, coagulase - positive *Staphylococcus aureus* was isolated from 30 cases (60%). Moreover, *Escherichia coli* from 8 cases (16%), *Klebsiella aerogenes* from 3 cases (6.0%), *Proteus vulgaris* from 6 cases (12.0%) and *Pseudomonas pyocyanea* from one case (2.0%) were also identified. The antibiogram of *Staphylococcus aureus* revealed that 96.7% were susceptible to sulfamethoxazole-trimethoprim followed by tetracycline (53.3%), cefotaxime (13.3%) and erythromycin (3.3%). It was found that 18, 27, 15, 20, 20, 30 and 24 isolates were susceptible to tetracycline, ceftriazone, cefalothin, erythromycin, cloxacillin, clove oil and garlic oil respectively. Majority of them were intermediate susceptible to ampicillin (70.0%) and resistant to cloxacillin (23.3%). The extracts of three medicinal plants: *Allium sativum* (kyet-thun-phyu) bulb, *Azadirachta indica* (tamar) leaves and *Eupatorium odoratum* (bizat) leaves by 97% ethanol, 50% ethanol and watery types were tested on the isolated *S. aureus* but showed no antibacterial activity. However, the antibacterial activity was obtained when tested with fresh juice of garlic and clove oil. The Minimum Inhibitory Concentration (MIC) of fresh garlic juice was 1.3 to 2% (v/v) and 1.3 to 5% (v/v) by agar plate dilution and test tube serial dilution methods respectively.

## INTRODUCTION

More than 100 years, *Staphylococcus aureus* remains a versatile and dangerous pathogen which plays an important role in sepsis and abscess formation. The frequencies of both community - acquired and hospital - acquired staphylococcal infections have increased readily, with little change in overall mortality. Treatment of these infections has become more difficult because of the emergence of multidrug-resistant strains [1]. *Staphylococcus aureus* had been recognized since 1883 as a common cause of infection and the

mortality rate was 82%. The organisms conjugate and share plasmids most frequently with organisms in the same environment, with similar cell walls [2]. A systematic review on seven randomized trials of the use of honey as a dressing for burns or wounds concluded that honey was effective [3]. Subrahmanyam (1996) reported that wound healing in honey treated patients occurred in 10 days in 275/450 patients and in 15 days in 350/450. The mean time for wound healing was 9 days. In conventional - treated patients wounds healed in 12-28 days with a mean time to wound healing of 13.5 days.

Moreover, wound infection occurred in 25 cases among honey - treated patients and 52 cases among conventional treated patients [4].

They also studied on the isolation of bacteria after honey application and demonstrated that 90% of the patient's wound became sterile after one week [5]. Antibacterial activity of plants was already reported by Mar Mar Nyein [6]. This study was emphasized on the effect of some plants' products on *S. aureus*, isolated from wounds of patients attended the Traditional Medicine Hospital and to record the antibiogram of *S. aureus*.

## MATERIALS AND METHODS

### *Patients studied*

A total of 50 cases (male = 25 female= 25) with septic wound (anapauk) attended the Traditional Medicine Hospital from 25 January to 26 April, 2005 were included. Patients who had received Western antibiotics or herbal medicine before two weeks of specimen collection were excluded.

### *Sampling*

After cleaning the outside areas of wounds, fluid with or without pus were collected by the aid of a syringe and placed in Stuart's transport media. The samples were carried to Bacteriology Research Division, Department of Medical Research (LM) through cold chain transportation.

### *Isolation, identification and characterization of pathogens*

Bacteriological testing was proceeded by standard method for isolation of bacterial pathogens, using Blood agar, Mannitol Salt agar, MacConkey agar, Nutrient agar and Ashdown agar. Gram stained reaction, biochemical reaction and serology were done for the diagnosis [7, 8].

### *Coagulase testing*

It was done by using slide and tube agglutination test by rabbit plasma [9].

### *Antibiotic susceptibility testing*

Based on the method described by Kirby-Bauer (1966) [10], antibiotic susceptibility testing was done using the discs of ampicillin (Oxoid – A - 10), sulphamethoxazole - trimetho-prim (BBL-SXT, 23.75:1.25), tetracycline (Eiken, Te-30) ceftriazone (BBL - Cef- 30), cefotaxime (DMR-Cfr-30), cephalothin (BBL-CF-30), erythromycin (BBL, E-15), and cloxacillin (BBL-Cx-1).

### *Plants and oils*

Three medicinal plants: *Allium sativum* (kyet-thun-phyu) bulb, *Azadirachta indica* (tamar) leaves and seeds, *Eupatorium odoratum* (bizat) leaves were extracted individually by 97% ethanol, 50% ethanol and watery types in Department of Traditional Medicine by standard methods. Clove oil (10µl/disc) from Sigma and freshly grinded juice of garlic oil respectively were used. All extracts and oil were impregnated in 8mm filter paper discs (Whatman No. 3) and dried at 42°C prior to the experiment.

### *Antibacterial susceptibility testing by agar disc diffusion*

It was done by the method of Kirby-Bauer, 1966 described in the previous experiments by Mar Mar Nyein [11].

### *Minimum Inhibitory Concentration*

It was done by agar plate dilution method or by test tube serial dilution method [12].

## RESULTS

### *Locality of patients*

Patients who came to the hospital were from different localities. Three different areas of Yangon were analysed and *Staphylococcus aureus* was isolated from 11 out of 15 cases from area 1, 7 out of 13 cases from area 2 and 5 out of 14 cases from area 3. Furthermore, it was isolated from 3 out of 5 cases who came from other cities (Table 1).

Table 1. Localities of the patients

Sr.No.	Localities of patients	Tested cases	Cases infected with <i>Staphylococcus aureus</i>
1.	Bahan Kyaukmyaung Yangon city Tamwe Kamayut Kyimyindine Pabedan	15	11 (73.33)
2.	Dagon myothit Yangon Okkalapa Shwepyithar Insein	13	7 (53.33)
3.	Thaketa Yangon Pazundaung Thingangyun Dawpon Kyauktan Hmawbi	14	5 (35.71)
4.	Dedaye Other cities Yenangyaung Rhakine Kyeikhto Paungde	5	3 (60.00)
5.	Unknown	3	3
	Total	50	30 (60.00)

Figures in parenthesis denote percentages

#### Site of infection

Wounds occurred in different sites and isolation of pathogens regarding to bacterial species is shown in Table 2. Out of 30 cases with *Staphylococcus aureus*, 12 cases were also infected with other pathogens such as *Proteus mirabilis*, *Klebsiella aerogenes* or with *E. coli*. *Escherichia coli* was isolated from 3 cases, *Klebsiella aerogenes*, *Proteus* and *Pseudomonas pyocyanea* were isolated from one case each.

#### Isolation of bacterial pathogens

Isolation of coagulase positive *Staphylococcus aureus* is shown in Table 3. A total of 30 strains were isolated from different age groups. Moreover, *E. coli*, *Klebsiella* spp., *Proteus* spp and *Pseudomonas pyocyanea* were isolated from 8 cases, 3 cases, 6 cases and one case respectively.

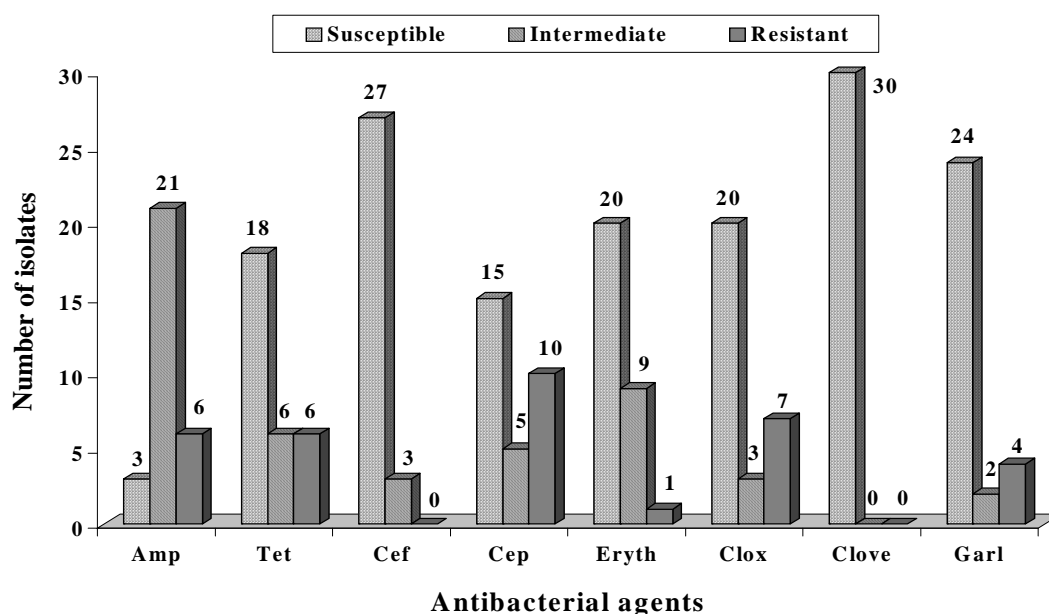
#### Antibiogram of *Staphylococcus aureus*

It was found that 96.7% were susceptible to sulfamethoxazole-trimethoprim followed

Table 2. Sites of infection in patients and isolation of *S. aureus* from patients

Sr. No.	Sites	Cases tested	No. of cases with pathogens	No. of cases infected with <i>S. aureus</i>	No. of cases infected with other pathogens
1	Shin	2	1	1	1=mixed with <i>Proteus</i> spp.
2	Foot	4	3	2	1= mixed with <i>Proteus</i> spp. 1=mixed with <i>Klebsiella</i> spp 1=with <i>E. coli</i>
3	Anus	9	6	6 (66.67)	1= mixed with <i>Proteus</i> spp. 1= with <i>E. coli</i>
4	Back	4	2	2	1= mixed with <i>Proteus</i> spp.
5	Big toes	4	3	2	1= with <i>Proteus</i> spp. 1= mixed with <i>Proteus</i> spp. 1= with <i>Klebsiella</i> spp
6	Maxilla	1	1	1	1=mixed with <i>E. coli</i>
7	Thigh	3	3	3	1=mixed with <i>E. coli</i>
8	Breast	9	4	4 ( 44.44)	1=mixed with <i>E. coli</i>
9	Buttock	1	1	1	
10	Sole	1	1	1	
11	Palm	1	1	1	
12	Knee	1	1	0	1= with <i>Pseudomonas</i> spp.
13	Wrist	1		1	
14	Neck	3	3	3	1=mixed with <i>E. coli</i> 1=mixed with <i>Klebsiella</i> spp.
15	Finger	1	1	0	
16	Inguinal	1	1	0	
17	Forehead, Backhead	2	1	1	
18	Cheek	2		1	1=with <i>E. coli</i> 1=mixed with <i>E. coli</i>
	Total	50	33	30 (60.00)	12=mixed with <i>Proteus</i> , <i>Klebsiella</i> or <i>E. coli</i> 3=with <i>E. coli</i> only 1= each with <i>Proteus</i> <i>Klebsiella</i> and <i>Pseudomonas</i> spp. only

Figures in parenthesis denote percentages



Amp= Ampicillin      Tet= Tetracycline      Cef= Ceftriazone      Cep= Cephalothin  
 Eryth= Erythromycin      Clox= Cloxacillin      Clove= Clove      Garl= Garlic

Fig 1. Effect of different antibiotics, clove oil and garlic on 30 isolates of *Staphylococcus aureus* isolated from 50 wound sepsis cases from Traditional Medicine Hospital

Table 3. Isolation of coagulase positive *Staphylococcus aureus* and other pathogens from different age groups of patients

Age Groups (years)	<i>Staphylococcus aureus</i>			Other pathogens			
	Male	Female	Both sexes	<i>E.coli</i>	<i>Klebsiella</i> spp.	<i>Proteus</i> spp.	<i>Pseudo-monas</i> spp
<20	2/2	1/1	3/3	1	0	1	0
20-30	1/4	3/6	4/10	1	1	1	0
31-40	3/5	0/2	3/7	1	1	3	0
41-50	2/5	8/10	10/15	3	0	0	1
51-60	3/4	3/4	6/8	1	0	1	0
>60	3/3	1/3	4/6	1	1	0	0
Total	14/24 (58.3)	16/26 (61.5)	30/50 (60.00)	16	6.00	12.00	2.00

Figures in parenthesis denote percentages

by tetracycline (53.3%), cefotaxime (13.3%) and erythromycin (3.3%). Moreover, 18, 27, 15, 20, 20, 30 and 24 isolates were susceptible to tetracycline, ceftriazone, cephalothin, erythromycin, cloxacillin, clove oil and garlic oil respectively. Majority

of them were intermediate susceptible to ampicillin (70.0%) and resistant to cloxacillin (23.3%). The extracts of *Azadirachta indica* (tamar) leaves, and *Eupatorium odoratum* (bizat) leaves by 97% ethanol, 50% ethanol and watery types showed no antibacterial activity when tested on the isolated *S. aureus*. However, the antibacterial activity was observed when tested with fresh juice of garlic and clove oil (Fig.1). The Minimum Inhibitory Concentration (MIC) of fresh garlic juice was 1.3 to 2% (v/v) by agar plate dilution method and the minimum bactericidal concentration of fresh juice was 1.3 to 5% (v/v) by test tube serial dilution method.

## DISCUSSION

Routes of transmission of bacterial species include air-borne transmission, implying dissemination throughout the institution and indirect transmission by fomites and agreed that proper sanitation is the necessity. Drug resistance and management of serious

*Staphylococcal* infection in hospitals were reported [13-15]. *Staphylococcus aureus* acquires resistance by mutation, conjugation, transduction or transformation, although transduction and transformation are rare resistance-acquisition methods for *Staphylococci* [16]. There were reports by using not only the antibiotics but also with natural products, such as honey and plant extracts. Comparison of wound healing after initial treatment with honey dressing and with silver sulphadiazine impregnated dressing by replacing dressings every two days revealed that honey is faster than silver sulphadiazine [5]. The present study showed that garlic extract was effective on *Staphylococcus aureus* by *in vitro* testing. However, the application in the topical use for treatment of wounds needs to be further elucidated. Antimicrobial activity of garlic, tea tree oil, and chlorhexidine against oral microorganisms was studied [17] and it was claimed that *Allium sativum* (garlic) (2.5%) and *Melaleuca alternifolia* (tea tree) oil (0.2%) might be an alternative to chlorhexidine. The present study revealed the importance of *S. aureus* in cases with wounds from TMH for the first time although its occurrence in other localities was also reported by many authors [18-21]. In this study, the finding that the fresh juice of garlic was more profoundly active than the extracted parts is also coincided with that of other workers [22, 23]. Compounds such as sulfur, alliin, allicin were known to possess antibacterial action, indicating their potential usefulness as antiseptic agents when necessary.

#### ACKNOWLEDGEMENTS

We would like to thank our Director-General Dr Paing Soe and Deputy Director-General Dr Soe Thein and Director Dr U Kyaw Moe for their keen interest and support. We would like to express sincere gratitude to Director-General Dr U Thein Swe, Department of Traditional Medicine and the Medical superintendent Dr U Sein Win from Traditional Medicine Hospital

and last, but not the least to patients who participated in this research.

#### REFERENCES

1. Lowy FD. *Staphylococcus aureus* infections. *New England Journal of Medicine* 1998; 520-532.
2. Smith TL & Jarvis WR. Antimicrobial resistance in *Staphylococcus aureus*. *Microbes and Infection* 1999; 795-805.
3. Bandolier. More on honey and wounds "Evidence based thinking about health care". <http://www.jr2.ox.ac.uk/bandolier/booth/alternat/honey2.html>.
4. Subrahmanyam M. Honey dressing for burns-an appraisal. *Annals of Burns and Fire Disasters* 1996; IX: 33-35.
5. Subrahmanyam, M, *et al.* Effect of topical application of honey on burn wound healing. *Annals of Burns and Fire Disasters* 2001; XIV: 143-145.
6. Mar Mar Nyein, Nwe Yee Win, Win Myint, Aye Aye Thein, Mi Mi Htwe, Win Win Maw & Aye Than, Plants possessing antibacterial activity. *Myanmar Health Sciences Research Journal* 1999; 1(1-3): 26-32.
7. WHO. Manuals of diagnostic laboratory procedure for acute enteric infection. Simplified methods *World Health Organization*, Geneva, 1990.
8. Ewing WH. *Edwards and Ewing's identification of Enterobacteriaceae*. 4th edition, Elsevier Science Publishing Co. Inc. New York, 1986.
9. Cowan ST. *Cowan and Steel's Manual for the identification of medical bacteria*. Second edition. Cambridge University Press, 1974.
10. Bauer AWW, Kirby WMM, Sherris JC & Turck M. Antibiotic susceptibility testing by standardized disc method. *American Journal of Clinical Pathology* 1966; 45: 493-496.
11. Mar Mar Nyein, Chit Maung, Mya Bwin & SJ Tha. *In vitro* testing of various indigenous plant extracts on human pathogenic bacteria. *Myanmar Health Sciences Research Journal* 1991; 3: 89-99.
12. Cruickshank, R. *Medical Microbiology*. 11<sup>th</sup> edition, UK. The English Language Book Society and Churchill Livingstone Ltd, 1968.
13. Schwarz K. *Burn wound infections* 2005; 13 pages. <http://www.emedicine.com/med/topic258.htm>.

14. Brumfitt W & Hamilton MJ. Methicillin resistant *Staphylococcus aureus*. *New England Journal of Medicine* 1989; 320: 1188-1196.
15. Sheagren JN. *Staphylococcus aureus* the persistent pathogen. *New England Journal of Medicine* 1984; 310: 1368-1373, 1437-1442.
16. Al-Masaudi SB, Day MJ & Russell AD. Antimicrobial resistance and gene transfer in *Staphylococcus aureus*. *Journal of Applied Bacteriology* 1991; 70: 279-290.
17. Groppo FC, Ramacciato JC, Simoes P, Florio FM & Sartoratto A. Antimicrobial activity of garlic, tea tree oil, and chlorhexidine against oral microorganisms. *International Dental Journal* 2002, 52(6): 433-437.
18. Khin San Myint Kyi. Bacteriological study of post-operative wound sepsis in surgical wards of Yangon General Hospital. M. Med.Sc (Microbiology), Institute of Medicine 1, 1998.
19. Meiji Soe Aung. A bacteriological profile of orthopaedic post-operative wound sepsis in Yangon General Hospital. M. Med. Sc (Microbiology), Institute of Medicine 1, 2004.
20. Mya Mya Lwin . Bacteriological study of community acquired infection. M. Med. Sc (Microbiology), Institute of Medicine 1, 2001.
21. Nang Nyo Nyo Win. A bacteriological study of common bacterial infections in cancer patients of Yangon General Hospital. M. Med. Sc (Microbiology), Institute of Medicine 2003.
22. Lee YL, Wang Y, Shanbrom E & Thrupp L. Antibacterial activity of vegetables and juices. Review article. *Nutrition* 2003, 19 (11/12): 994-996.
23. Onyeagba RA, Ugbogu OC, Okeke CU & Iroakasi O. Studies on the antimicrobial effects of garlic (*Allium sativum* Linn.) ginger (*Zingiber officinale* Roscoe) and lime (*Citrus aurantifolia* Linn.). *African Journal of Biotechnology* 2004; 3(10): 552-554.