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FOUR ANTIBIOTICS SENSITIVITY TO MICROORGANISMS IN LOCAL WOUND INFECTION

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Abstract

The susceptibility to antibiotics of microorganisms isolated from various wounds and burns infections of the patients in Nakorn Chiang Mai Hospital were investigated. The antibiotics sensitivities test was performed by serial tube dilution method to four antibiotics; Soframycin (Framycetin sulphate), Garamycin (Gentamycin), Terramycin (Tetracycline), and Colimicin (Colistin methane sodium). From 79 specimens, the isolated organisms were **Staphylococcus aureus** (34 strains), **Escherichia coli** (10 strains), **Proteus mirabilis** (8 strains), Klebsiella (7 strains), Pseudomonas aeruginosa (7 strains), and Enterobacter (4 strains) respectively. The results indicated that 85% of the isolated strains of Pseudomonas aeruginosa were sensitive to garamycin and colistin, and 25% were shown to be resistant to Soframycin. In case of **Staphylococcus aereus**, 97.5 %, 91.4 %, 61.8 % and 61.8 % were sensitive to garamycin, soframycin, terramycin and colistin respectively. Almost all strains of **Proteus mirabilis** were resistant to all antibiotics. All strains of **Klebsiella** were sensitive to garamycin and only 42.8 % to the other three antibiotics. **Enterobacter** were found sensitive to garamycin, soframycin, colistin and terramycin at the percentage of 75, 50 and 25 respectively.

Introduction

Antibiotics have been successfully employed in the treament of infections disease. The feasibility of using antibacterial substances **in vivo** as chemotherapeutic agents depends primarily upon the specific action of such substances, regarding to **selective action** on the microorganisms

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without significant harmful effects on the host (3). The microorganisms differ markedly in resistance and sensitivity to the various antibiotics, not only between genera and species but also between strains of the same species. They may produce enzyme to neutralize antibiotic such as Penicillinase in **Staphylococcus aureus** which can inactivate penicilline (2). Some of them have mutate into resistant strains to some antibiotics. However, it is not neccessary to test the sensitivity of some organisms such as **Diplococcus pneumonia**, **Staptococcus pyogenes**, **Neisseria meningitidis, and Neisseria gonorrhea** (12). Because they are always sensitive to penicillin.

The wound infection is usually caused by contamination of serrounding bacteria which includes bacteria from skin, clothes and dust. (4) Staphylococcus aureus is the most frequently found in this case, the other include **Streptococcus pyogenes** (Lancefield's group A), Spore forming bacilli, (Aerobic and Anaerobic) **Enterococci**, hemolytic and non-hemolytic streptococci, **Diphtheroids**, Gram negative intestinal bacilli, **Pseudomonase aeruginosa**, **Proteus Pasturella septica** (5).

In case of post operation the inflamation of wound is mostly caused by contaminative **Staphylococcus aureus** from air, nasal cavity of staff hospital, or from skin, clothes, and blankets of the patients (6).

The patients with burn infection are frequently found infected with **Pseudo monas**, **Staphylococcus**, **Sterptococcus phyogenes**. Other organisms less frequently found in burn infections are **Proteus**, **Klebsiella** and **Enterobacter.** (7)

Baber et al (2) performed an experiment of Bacterial resistance to antibiotic, finding that the number resistance strains of microorganisms to antibiotic will increase with the period of that antibiotic used. It is then important that susceptibility of pathogenic organisms isolated from patients be done to guide to the selection of proper antibiotic for treatment.

Material and Method

1. Specimens and organisms

The specimens were swab from inflamation area both superficial and deep, from operative incisions and burns from patients at O. P. D. of Nakorn Chiang Mai Hospital. A fresh swab was inoculated into the following media; Blood agar, Mc. Conkey agar, EMB and Thioglycolate broth (for anaerobic organisms). The transport media was tryptricase soy broth with sterile cotton swab in 10 ml. test tube. The isolated microorganisms from specimen were identified by using biochemical test. (Bailey & Scott (1)). The pure cultures were preserved in

trypticase soy agar, and stored at room temperature or refrigerator.

II. Antimicrobial agents

Four antibiotics were used in the experiment :

1. Soframycin 2. Colimycin 3. Terramycin and 4. Garamycin. These antibiotic were dissolved in sterile distilled water to make concentration being 2000 mcg or unit / ml and divided them to 4 ml. in each bottle store in deep freeze for stock solution. Solution was thawed and it was diluted to 200 mcg. or unit/ml. for using in the test, and the remaining stock solution from each test was discarded.

III. Susceptibility testing method

Stock solutions of antimicrobial agents were thawed and diluted with sterile distilled water, the final concentration being 200 mcg or unit/ml. The serial dilution of antimicrobial was done using trypticase soy broth as diluent to make concentration of 100, 50, 25, 12.5, 6.25. 3.125, 1.562, 0.781 and 0.36 mcg or unit / ml., the final volume being 0.5 ml. Then 0.5 ml of 1 : 1000 dilution of overnight culture of organisms in trypticase soy broth was added to each antimicrobial dilution. Incubate 37°C overnight and read MIC (Minimal Inhibitory Concentration).

Results

The total of 79 strains of various organisms were isolated from wound infections as shown in **table 1. Staphylococcus aureus** was the predominant microorganisms. The Minimal Inhibitory Concentration of each organisms to four antibiotics were shown in **table 2.** with their MIC standard of antibiotics shown in **table 3.** The percentage of sensitive, intermidiate, and resistant of organisms to antibiotics are shown in **table 4.** Most strains of **Staphylococcus aureus**, **Pseudomonas aeruginosa**, **Proteus mirabilis**, **Klebsiella** and **Enterobacter** are sensitive to garamycin. Most of the strains of these microorganisms are resist to terramycin.

Discussion

The determination of susceptibility of pathogenic organisms to antibiotic is very important in the selection of antibiotic for therapy. The organisms are different in their susceptibilities to antibiotics. There are many methods determine susceptibility of organisms to drugs, but the quantitative accuracy one is serail tube dilution method. Many factors such as; pH of media, incubation time, inoculum size, and stability of drugs (8), may influence in the detection of minimal inhibitory concentration of each drug.

From many report (9, 10) we found that the strains of **Staphylococcus aureus** isolated from hospitals resist penicillin.

These incidences have been attributed to the fact that large-scale use of penicillin in the hospital has wiped out the penicillin sensitive **Staphyloccus aureus;** thus remaining only resistant variants (usually producing penicillinase). A similar situation has accured to gram-negative organisms, especially hospital strains. The excessive use of drug lead to suppression of drug susceptible microorganisms and favors the survival of drug resistant ones such as; **Aerobacter, Proteus, Pseudomonas** species.

From the results it is expected that garamycin and soframycin are excellent for the treatment of local wound infection. Although soframycin, only has intermidiate sensitivity effect on strains of **Pseudomonas aeruginosa.** Salcida and coworker(30) reported, however soframycin was the best drug for the treatment of corneal infection cause by **Pseudomonas aeruginosa** without any harm to normal ocular tissue.

Most of strains of tested organisms were not sensitive to terramycin. These might be due to the wide spread use of terramycin resulting in elimination of tetraccycline sensitive organisms.

In addition soframycin and garamycin are active against gram-positive cocci and gram-negative bacteria including penicillin resistance Staphylococci. It is therefore useful in the treatment of mixed organisms in wound infection. Both soframycin and garamycin are relatively new antibiotic, so the percentage resisting organisms is not very high.

Table 1

79 Isolated Organisms from various wounds, Abscesses and Burns.

Isolated Microorganisms	No. of Isolated
Staphylococcus aureus	34
Escherichia coli	10
Proteus mirabilis	8
Pseudomonas aeruginosa	7
Klebsiella	7
Enterobacter	4
B-Hemolytic Streptococci	6
Gram-negative Anaerobic Bacilli	3
Total organisms	79

Table 2

Minimal Inhibitory Concentration (MIC) of 79 strains of Isolated organisms to four antibitics.

Organisms	Garamycin M I C (mcg/ml)	Colistine M I C (mcg/ml)	Terramycin M I C (mcg/ml)	Soframycin M I C (mcg/ml)
34 Strains of Staphylococcus asreus	0.39/22*	100/4	0.39/19	0.39/8
	0.78/8	> 100/30	0.78/12	0.78/9
	1.56/3		3.125/2	1.56/6
	3.125/1		12.5/1	3.125/8
			25.0/2	6.25/2
			50.0/7	25.0/1
			> 100/1	
10 Strains of Escherichia Coli	1.56/1	6.25/1	1.56/3	6.25/1
	3.125/5	12 5/1	12.5/1	12.5/3
	6.25/2	25.0/1	> 100/6	25.0/5
	12.5/1	50/1		50.0/1
		100/2		
		> 100/1		
8 Strains of Proteus mirabilis	0.39/1	> 100/8	3.125/1	3.125/1
	1.56/1		12.5/1	6.25/1
	6.25/3		100/5	25.0/5
	12.5/3		> 100/1	50.0/1
7 Strains of Pseudomonas aeruginosa	0.39/1	25.0/2	1.56/1	6.25/3
	0.78/3	50.0/2	3.125/2	12.5/1
	1 56/3	100/2	6.25/3	25.0/2
	100/1	> 100/1	100/1	> 100/1
7 Strains of Klebsiella	0.39/2	25/3	0.78/3	3.125/3
	0.78/3	> 100/4	3.125/1	6.25/3
	1.56/2		12.5/1	100/1
			50.0/1	
			> 100/1	

(continue)

Organisms	Garamycin M I C (mcg/ml)	Colistin M I C (mcg/ml)	Terramycin M I C (mcg/ml)	Soframycin M I C (mcg/ml)
4 Strains of Enterobacter	0.78/1	50.0/1	0.78/1	3.125/2
	1.56/1	> 100/3	6.25/1	6.25/1
	50.0/1		25.0/1	100/1
			100/1	

* 0.39/22 = 22 strain of Staphylococcus aureus has MIC = 0.39 mcg/ml

Table 3

Showing the standard limit of Minimal Inhibitory Concentration for "Sensitive", "Intermediate", and "Resistant" strains.

Antibiotics	Sensitive (mcg/ml)	Intermediate (mcg/ml)	Resistant (mcg/ml)
Gentamycin	≤ 2.5	> 2.5–10	> 10
Colistin	≤ 100 u/ml	> 100–500 u/ml	> 500 u/ml
Tetracyclin	≤ 2.5	> 2.5–5.0	> 5.0
Streptomycin*	≤ 5.5	> 5.0–25	> 25

*Streptomycin have Chemical Semilar to Soframycin.

Table 4

Antibiotic Susceptibility of Pathogenic organisms Isolated from various wounds and burns.

Percentage of sensitive, Intermediate, and Resistant.

Isolated org.	No. of strains	Garamycin mcg/ml			Colistin unit/ml			Terramycin mcg/ml			Soframycin mcg/ml		
		% S	% I	% R	% S	% I	% R	%S	% I	% R	% S	% I	% R
Staphylococcus aureus	34	97.5	2.5	0	11.8	88.2	0	61.8	11.8	26.4	94.1	5.9	0
Escherichia coli	10	10	80	10	50	50	0	30	0	70	0	90	10
Proteus mirabilis	8	25	37.5	37.5	0	100	0	0	12.5	87.5	12.5	50	37.5
Pseudomonas aeruginosa	7	85.8	0	14.2	85.8	14.2	0	14.3	42.8	42.8	0	85.8	14.2
Klebsiella	7	100	0	0	42.8	57.2	0	42.8	14.3	42.8	42.8	42.8	14.3
Enterobacter	4	75	0	25	25	75	0	25	0	75	50	25	25

S = Sensitive

I = Intermediate

R = Resistant

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