BODY OF REPORT

SEATO Medic Study No. 66	Antibiotic Sensitivities and Cross Resistance Patterns of Pathogenic Bacteria Indigenous to Thailand
Project No. 3A 025601 A 811	Military Medical Research Program S. E. Asia
Task 01:	Military Medical Research Program S. E. Asia
Subtask 01:	Military Medical Research Program SEASIA (Thailand)
Reporting Installation:	US Army-SEATO Medical Research Laboratory APO San Francisco 96346 Division of Medical Research Laboratories Department of Bacteriology and Immunology
Period Covered by Report:	1 April 1964 to 31 March 1965
Principal Investigator:	Howard E. Noyes, Ph.D.
Associate Investigators:	Mrs. Panyasri Benjadol Charas Yamarat, M.D.
Assistant Investigators:	Miss Kannikar Punyashthiti Miss Pannee Nithi-uthai
Reports Control Symbol:	MEDDH-288
Security Classification:	UNCLASSIFIED

Objective: To determine bacteriostatic levels of commercially available and experimental chemotherapeutic agents for pathogenic microorganisms of importance to SEATO military units and to residents of Thailand. Pyogenic staphylococci from wounds and respiratory pathogens have been tested during this testing period because of their frequence of occurrence and their obvious military importance. The use of antibiotics to moderate bacterial respiratory diseases and to prevent or cure wounds is related directly to the susceptibility of the etiologic agents. The problem of antibiotic-resistant organisms could be especially critical in Thailand where antibiotics are available without prescription, and, in some instances, are relatively inexpensive. The purpose of this phase of this study was to determine

bacteriostatic levels of a number of antibiotics for recent isolates of respiratory pathogens and for pyogenic coagulase-positive staphylococci.

Description: All organisms in this study were isolated from patients at Children's Hospital or Women's Hospital, Bangkok, Thailand. Respiratory pathogens were from four groups, based on the diagnosis entered on the chart by the attending physician. These were (1) patients with pyrexia of unknown origin and were selected from those out-patients who had no sign of respiratory disease except fever (2) patients from the out-patient department with upper respiratory infections (3) patients hospitalized with respiratory diseases and (4) patients hospitalized for reasons other than hemorrhagic fever or respiratory diseases. Media used for isolation of respiratory pathogens consisted of mannitol salt agar, McConkey agar, Mueller-Hinton agar, sheep blood agar, chocolate agar, blood tellurite agar and trypticase soy agar and broth. Plate sensitivity tests with the use of antibiotic disks were performed for all pathogenic isolates by using appropriate media for each organism. The concentration of the antibiotic disks used were 5 units for penicillin, 5 mcg for erythromycin and streptomycin and 10 mcg for tetracycline, kanamycin, novobiocin, neomycin and chloramphenicol.

Coagulase-positive staphylococci consisted of 235 strains isolated from 532 specimens obtained from the surgical ward and the minor operating rooms in the Women's Hospital or Children's Hospital, Bangkok, Thailand. There were 170 strains from wounds, 39 strains from the anterior nares of doctors and nurses; 16 strains from anterior nares of patients and 10 strains from fomites. All organisms were tested for sensitivities to penicillin G, methyl phenyl isoxazolyl penicillin D-alpha amino benzyl penicillin, sodium dimethoxy phenyl penicillin, oxytetracycline, 6-methylene oxytetracycline, chloramphenicol and streptomycin. Sensitivity tests were carried out in trypticase soy broth, pH 7.4 by use of the tube dilution method. The inoculum for each tube was approximately 10^6 organisms/ml and the bacteriostatic level was the lowest concentration of antibiotic preventing visable growth.

Progress: Potentially pathogenic respiratory pathogens were isolated from 92.3 of 195 patients regardless of their group. If one arbitrarily chooses bacterial counts of 10^3/swab of a potential pathogen as significant, 73 of the 195 patients had 2 or more pathogenic species present at the time of sampling. There were no significant differences among groups in terms of bacterial counts of numbers of bacterial species isolated.

Percentages of the organisms sensitive to selected antibiotics can be seen in Table 1. These date indicate that of those tested the most effective antibiotic for coagulase positive staphylococci was chloramphenicol followed in decreasing order of effectiveness by tetracycline, erythromycin, neomycin, kanamycin, penicillin and streptomycin. Chloramphenicol was the most effective for Hemophilus influenzae, beta hemolytic streptococci, and Corynebacterium diphtheriae but erythromycin was the best antibiotic for Diplococcus pneumoniae and Neisseria meningitidis. Klebsiella pneumoniae was most sensitive to kanamycin and neomycin

Table I

ANTIBIOTIC SENSITIVITIES OF PATHOGENIC BACTERIA ISOLATED FROM THROAT SWABS

Organism	No. strains isolated	No. of strains sensitive to Penicillin 5 units	Erythromy. 5 mcg.	Streptomy. 5 mcg.	Tetracyc. 10 mcg.	Kanamy. 10 mcg.	Novobiocin 10 mcg.	Neomycin 10 mcg	Chloromy. 10 mcg.
Beta hemoytic streptococci	70	65	69	48	70	37	61	33	70
D. pneumoniae	67	65	67	51	56	17	65	24	63
S. aureus	110	66	97	61	100	76	110	86	110
H. influenzae	70	58	69	62	68	69	65	67	70
N. meningitidis	29	24	29	22	26	29	28	23	26
C. diphtheriae	39	35	38	35	36	27	29	27	39
K. pneumoniae	15	0	0	10	13	15	5	15	11

Table II

PERCENTAGES OF SENSITIVE STAPHYLOCOCCI TO EIGHT ANTIBIOTICS AS CLASSIFIED BY SOURCES

Coagulase positive S. aureus from	Total	Percentage of strains sensitive to 6.25 mcg/ml Pencicillin G	Prosta-phylin	Pentrexyl	Staph-cillin	Metha-cycline	Terra-mycin	Chloram-phenicol	Strepto-mycin
Patients	170	44.1	92.9	68.2	83.5	89.4	80	13.5	11.8
Doctors & nurses noses	39	48.7	100	97.9	100	92.3	79.5	41.0	33.3
Fomites	10	40.0	100	90.0	90.0	60.0	40.0	30.0	30.0
Patients' noses	14	28.6	93.9	93.9	93.9	71.4	28.6	14.3	21.4

followed by tetracycline. The laboratory data related well to clinical data in that most of the hospitalized patients treated with the antibiotics indicated by in vitro studies responded favourably. Most out-patients treated didn't return for further treatment and it was assumed the antibiotics given were also effective for these patients.

Coagulase-positive staphylococci were isolated from 63.2 percent of wounds, 30.7 percent the external nares of patients, from 54.9 percent from the external nares of hospital personnel and only 7.1 percent from the 140 fomites sampled. Results in the Table II show the percentages of sensitive staphylococci to eight antibiotics as classified by sources. There was a high incidence of patients' strains resistant to chloramphenicol, streptomycin, and penicillin G, but most strains were sensitive to the other three penicillins and two tetracyclines tested. Rates of strains sensitive to penicillin G, methyl phenyl isoxazolyl penicillin, d-alpha amino benzyl penicillin, chloramphenicol and streptomycin in carriers were higher than strains isolated from patients. Strains from fomites and patients' noses were less sensitive to tetracycline than strains from wounds or hospital personnel.

In the evaluation of new antibiotics, the possibilities of cross-resistance with existing antibiotics must be considered. Antibiotics which have common structural nuclei tend to have patterns of cross-resistance. There was some cross-resistance among penicillin G and the biosynthetic penicillins. An important cause of resistance to penicillin G is the elaboration of penicillinase by resistant organisms. The only one of these new penicillins sensitive to penicillinase was d-alpha amino benzyl penicillin. Its sensitivity pattern predictably paralleled that of penicillin G. It was noted that while the two tetracyclines had similar resistance patterns, most strains were more sensitive to 6-methylene exytetracycline than to oxytetracycline.

The high rate of chloramphenicol resistance of strains isolated from wounds and the low rate of resistance of strains from nares was unexpected. One explanation seems to be the extensive usage of chloramphenicol in patients hospitalized for long periods whereas none of the patients in the respiratory disease study had received antibiotics for the current illness. Among patients receiving chloramphenicol it was found that 34 of 35 strains were resistant to that drug.

Summary: Antibiotic sensitivity studies of respiratory pathogens isolated from 195 patients showed that chloramphenicol was the antibiotic of choice insofar as sensitivity for these patients until laboratory sensitivity studies were available. In most instances there was a positive correlation of in vitro sensitivity, choice of antibiotic by the attending physician and clinical response of the patient. Studies on antibiotic sensitivity of coagulase-positive staphylococci from wounds, the anterior nares of patients and hospital personnel, and fomites indicated that methyl phenyl isoxazolyl penicillin was most effective followed in order by sodium dimethoxy phenyl penicillin, 6-methylene oxytetracycline HCl, d-alpha amino benzyl penicillin, oxytetracycline, penicillin G, chloramphenicol and streptomycin.

The incidence of resistance to chloramphenicol and streptomycin was more than 80 percent while only 54 percent of the strains were resistant to penicillin G. More than 75 percent of all strains were sensitive to the remaining antibiotics.

Conclusion: Most known respiratory bacterial pathogens are present in Thailand and as such constitute a potential military problem. The importance of definitive laboratory studies for patients with respiratory complaints is emphasized by the fact that clinical symptoms alone were inadequate for diagnosis. In the absence of laboratory support, the broad spectrum antibiotics would be the drugs of choice. The drugs of choice for wounds infected with coagulase positive staphylococci would be one of the penicillinase refractory penicillins or a tetracycline derivative.

Publications: Noyes, H.E., Benjadol, P., Thareesawasdi, M., and Senadisai, P., Antibiotic Sensitivities and Cross-Resistence Patterns of El Tor Vibrios and other Enteric Pathogens Indigenous to Thailand. Antimicrobial Agents and Chemotherapy 1964. In press.