

SEATO MEDICAL RESEARCH STUDY ON ANTIMICROBIALS

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Period of Report: 1. April 1965-31 March 1966

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Title: Antibiotic Sensitivities and Cross Resistance Patterns of Pathogenic Bacteria Indigenous to Thailand

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Objective - The objective of these studies is to determine the bacterial flora of infections of potential importance to military personnel in Southeast Asia and to determine antibiotic sensitivities of the causal agents. In the past studies have been carried out on organisms responsible for diarrheal diseases, respiratory diseases, urinary infections and staphylococci isolated from post-operative wounds. During the past year studies have been concerned with determining the antibiotic sensitivities of the microbial flora of infected traumatic wounds of patients at Police Hospital, Bangkok, Thailand and at the Provincial Hospital in Udornthani, Thailand. In addition a study on the effectiveness of topical antimicrobials as adjuncts to systemic antibiotic therapy of war wounds was carried out in Saigon, Republic of Viet Nam.

Description - Wound cultures were taken by inserting standard machine rolled swabs deep into the wounds until the swabs were saturated. The cotton tips were broken off into screw-capped vials containing 0.9 ml of 1.0 percent trypticase at pH 7.0. The streak plate technique was used for making quantitative estimates of different bacterial species. The following culture media were used: MacConkey agar for enterobacteriaceae, mannitol salt agar for staphylococci, SF medium for enterococci and blood azide egg yolk medium for clostridia. Commercial disks were used to determine antibiotic sensitivities of bacterial isolates.

The study on topical antimicrobials was carried out at Tong Y Vien Cong Hoa, a 1500 bed Army of Viet Nam hospital on the outskirts of Saigon. Initial removal of casualties was by litter, sampan, or jeep. Since there were no battle lines, first echelon care was variable, i.e. at aid stations, at provincial hospitals or at Cong Hoa itself, where the routine procedure was as follows:

Most patients arrived at Cong Hoa by helicopter and an ambulance took them to the triage room within minutes. After an immediate physical examination the patients were moved to the resuscitation room where treatment was initiated. In most instances the patient then received a single intramuscular injection containing penicillin, streptomycin, morphine, camphor, nikethamide, tetanus antitoxin and vitamin K. Blood was drawn for cross-matching and, if indicated, an X-ray was taken. If indicated, an infusion was started and the patient remained in the operating room until he was taken to surgery. If he did not go to surgery, he remained in the resuscitation room until his condition was stable enough that he could be sent to a ward. Operated patients were taken to the recovery/intensive care suite where they remained until they could be sent to a ward.

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When a patient went to the ward he was usually ambulatory and little active nursing care was needed. Each surgical pavilion had about 150 patients with 6 to 10 personnel to administer all nursing care and ward maintenance. Nearly every patient got a daily dressing change either in the ward or in the dressing room. All of the patients in this study received daily intramuscular injections of 1.2 megaunits of aqueous procaine penicillin G and 1.0 gram of streptomycin sulfate for at least the first 8 days of hospitalization. Initial wound swabs for qualitative and quantitative bacteriology were taken in the resuscitation room and on the 3rd, 6th and 9th days of hospitalization but topical therapy was not started until the 2nd day of hospitalization.

The antimicrobial regimen received by the patient was decided by his order of admission. The study was carried out in two parts. In part A, which was carried out in August, 1965, two antimicrobial preparations were studied. One was mafenide, also known as Marfanil and Sulfamylon^{(R)*} Chemically it is 4-(aminomethyl benzenesulfonamide hydrochloride) and it is different from other sulfa drugs in that it is not inhibited by the p-amino benzoic acid (PABA) present in most wounds. It was used as a 10 percent solution of Sulfamylon^(R) hydrochloride and sprayed on each wound daily for 15-30 seconds. The other drug was Polybactrin,^{(R)**} a commercial mixture of neomycin sulfate, zinc bacitracin and polymyxin B sulfate supplied as a micronized dry powder. (Table 1). Part B was carried out in September and October, 1965. Antimicrobials used were Sulfamylon^(R) and Neosporin,^{(R)***} a commercial preparation containing different ratios of the same peptide antibiotics in Polybactrin.^(R) These were applied twice daily at which times bandages were changed.

Progress - Specimens from infected traumatic wounds were obtained from 86 patients in Udornthani and 87 patients in Bangkok. The organisms isolated most frequently were Staphylococcus aureus and Pseudomonas sp. Other organisms isolated in order of decreasing frequency were coliforms, enterococci, Staphylococcus epidermidis, Proteus sp. and Clostridium perfringens. Antibiotic sensitivity studies showed that most isolates of S. aureus and S. epidermidis were resistant to penicillin G and tetracycline while about half were sensitive to novobiocin and erythromycin. Most Pseudomonas sp. were sensitive to polymyxin B and neomycin; about half were sensitive to kanamycin and almost all were resistant to streptomycin, chloramphenicol, colimycin, and tetracycline.

In the study carried out in Viet Nam there were many variables which complicated statistical evaluation of the data. Examples are severity of wounds, time between wounding and admission to the hospital, blood loss, degree of shock, and inability to sample every wound at each sampling time. Comparability of anatomical distribution of wounds in treatment groups can be seen in Table 2 which indicates that the distribution of wounds and the average number of wound per patient were reasonably consistent.

For the purposes of this study a count of 10^3 or more of viable bacteria per ml. of wound exudate constituted contamination of the wound and organisms countable at 10^6 or more/ml were considered to be responsible - at least in part - for the infectious process. When one considers the wounds in Study A having countable organisms at 10^3 or more/ml of exudate, the bacteria most frequently present at initial sampling in order of decreasing frequency were Staphylococcus aureus, Staphylococcus epidermidis, coliforms (lactose-fermenting enterobacteriaceae), and Pseudomonas sp. Proteus sp. and clostridia were found less frequently and not in all groups. The following changes were noted during the 9 days of observations. Percentages of wounds contaminated with S. aureus decreased about 15.18 percent in all group except the one receiving no topical therapy, whereas the decrease of wounds contaminated by S. epidermidis was much more pronounced in the two groups receiving topical antimicrobials. Percentages of wounds contaminated by coliforms were decreased only in the group treated with Polybactrin^(R). Percentages of fecal streptococci decreased all groups except the one receiving no topical

therapy and Pseudomonas sp. increased in all groups. Clostridia were found infrequently and were rarely present in a given wound at all sampling times. Proteus sp. was present in wounds of all groups but never tended to become the dominant organism. Beta hemolytic streptococci were rarely seen.

Considering wounds containing 10^6 or more/ml of wound exudate if the group treated with topical Polybactrin^(R) contained markedly fewer S. aureus and coliforms by the ninth day. Pseudomonas sp. persisted and increased in all groups although increasing somewhat more slowly in the Polybactrin^(R) group. Enterococci persisted in all wounds throughout the testing period. S. epidermidis Proteus sp., and clostridia were present in too few patients to permit comparisons.

Saline controls were deleted in Study B. At the 10^3 or more/ml level there were appreciable decreases of S. aureus, S. epidermidis, coliforms, and enterococci in the Neosporin^(R) group but counts of Pseudomonas sp. and Proteus sp. were not influenced appreciably. At the 10^6 or more/ml level there were decreases in the percentages of wounds infected by S. aureus, coliforms, and enterococci. As in Study A, Proteus sp., S. epidermidis and clostridia were not regularly present in wounds at the 10^6 /ml level and did not constitute a serious problem compared to the pathogens above.

Results of antibiotic sensitivity tests are shown in Table 3. Most of the organisms tested were sensitive to one or more of the three peptide antibiotics studied. Additional studies not shown in the table indicated that 16 of 134 strains of S. aureus and 46 of 57 strains of clostridia were sensitive to penicillin. It is possible that sensitivity to the routinely administered penicillin is an explanation for the paucity of clostridia seen in these wounds. Unexplained, however, was the rarity of clinical gas gangrene in those few patients with high counts of Clostridium perfringens or Clostridium bifermentans. Laboratory studies showed that most isolated pure cultures of C. perfringens were toxigenic in vitro and pathogenic for guinea pigs.

Summary.

1. Studies of the bacterial flora of infected traumatic wounds in Bangkok, and Udonthani, Thailand indicate that the causal agents are usually penicillin resistant S. aureus and/or Pseudomonas sp.

2. Studies were carried out to determine the effect of topical antimicrobials on the bacterial flora of war wounds of Vietnamese soldiers. The topical antimicrobials were in addition to the systemic penicillin and streptomycin used routinely. The qualitative and quantitative bacterial flora of patients treated with Sulfamylon^(R) (mafenide) changed very little when compared with the flora of untreated wounds. Treatment with two different commercial preparations of mixtures of neomycin, bacitracin and polymyxin B resulted in decreases of S. aureus, coliforms and enterococci. None of the antimicrobial regimens tested prevented proliferation of Pseudomonas sp.

* Mafenide, in the form of Sulfamylon hydrochloride was supplied by Winthrop Laboratories, Rensselaer, New York through the courtesy of Dr. William P. Blackmore, Associate Director, Division of Clinical Research.

** Polybactrin was supplied by Calmic Limited of Toronto, Canada through the courtesy of Mr. D.K. Coleman.

*** "Neosporin" Aerosol was supplied by Dr. S.W. Singleton, Medical Department, Burroughs Wellcome & Co. (U.S.A.) Inc. Tuckahoe, N.Y.

Table I. Topical Therapy of War Wounds

Study A

Group	Topical Antimicrobial	Individual Application (once daily)	
		Amount	Volume
1	Polybactrin Neomycin Polymyxin B Bacitracin	25 mg 5000 units 1250 units	Dry Spray
2	None		
3	Sulfamylon	300-500 mg	3.5 ml
4	Physiological Saline	0	3.5 ml

Study B

Group	Topical Antimicrobial	Individual Application (twice daily)	
		Amount	Volume
1	Neosporin Neomycin Polymyxin B Bacitracin	5 mg 5000 units 400 units	Dry Spray
2	Sulfamylon	300-500 mg	3.5 ml
3	None		

Table II. Distribution of Wounds

Study A

Group	No. of Patients	No. of Wounds	Average No. Wounds/Patient	Percentage of Wounds of				
				Legs & Feet	Thigh & Buttock	Arms & Hands	Trunk	Neck & Head
Polybactrin	22	30	1.4	43.3	23.3	20.0	6.7	6.7
No topical Rx	27	41	1.5	46.3	24.4	22.0	2.4	4.9
Sulfamylon	32	45	1.4	51.1	13.3	20.0	13.3	2.2
Saline	27	35	1.3	37.1	31.4	22.9	5.7	2.9

Study B

Group	No. of Patients	No. of Wounds	Average No. Wounds/Patient	Percentage of Wounds of				
				Legs & Feet	Thigh & Buttock	Arms & Hands	Trunk	Neck & Head
Neosporin	44	49	1.1	44.9	30.6	18.3	6.1	0
Sulfamylon	26	29	1.1	48.2	10.3	27.6	13.8	0
No topical Rx	67	81	1.2	58.0	19.8	19.8	2.5	0

Table 3. Antibiotic Sensitivities of Bacteria Isolated from War Wounds in Viet Nam

Organism	Number Isolates Tested	Number Sensitive to		
		Bacitracin (10 unit) disk	Polymyxin B (300 unit) disk	Neomycin (30 mcg) disk
<u>Staphylococcus aureus</u>	233	216	29	224
<u>Pseudomonas sp.</u>	214	39	214	214
<u>Clostridium sp.</u>	49	39	0	0
Lactose positive enterobacilli (coliforms)	55	0	55	43
<u>Proteus sp.</u>	29	1	2	28