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GRAM NEGATIVE BACILLI IN BURNS

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ABSTRACT

In a period of nine months, 225 strains of Gram-negative bacilli isolated from burns were identified by a variety of tests in two burn centers in Tehran. The most common species were *Pseudomonas aeruginosa, Pseudomonas fluorescens, Escherichia coli, Enterobacter agglomerans, Enterobacter cloacae* and *Klebsiella pneumoniae*. Many strains of acinetobacter spp, providencia spp, proteus spp, serratia spp, and *Citrobacter freundii* were also isolated.

All the strains were tested for sensitivity to bacitracin, carbenicillin colistin, cephalothin, chloramphenicol, gentamicin, kanamycin, lincomycin, trimethoprim and tetracycline. The proportion of strains sensitive and resistant to different antibacterial agents varied widely with species of bacteria. Most of the strains except pseudomonas spp were sensitive to few of the antibacterial agents and posed no problem with relation to treatment. Pseudomonas spp. were 100% sensitive to polymyxin B and colistin (polymyxin E) and 90% resistant to gentamicin and carbenicillin.

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INTRODUCTION

In the early 1940's, infections caused by Gram-positive organisms were frequent following burn injuries and they often resulted in a rapidly fatal course.

After the introduction of penicillin and penicillinase-resistant penicillins and broad-spectrum antibiotics which controlled infections caused by Gram-positive organisms, Gram-negative organisms rapidly became the most prominent pathogens.[1]

Gram-positive organisms are prevalent initially in burns but are gradually suppressed by the Gram-negative opportunists that appear to have greater propensity to invade.[2]

Following the successful control of infection with *Pseudomonas aeruginosa*, other Gram-negative bacilli have become a more common cause of septicemia and death in severely burned patients.[3]

We report the identification and antibiotic sensitivity patterns of a series of Gram-negative bacilli isolated from burns in two burn hospitals over a period of eight months.

MATERIAL AND METHODS

Methods of collection

The quantitative swab culture was obtained by twirling the end of a sterile cotton-tipped applicator on a one square centimeter area of the burn wound for five seconds. Sufficient pressure was applied to the tip of the swab to cause minimal bleeding in the underlying tissue. The swab tip was then broken off into a sterile tube containing 3 ml of thioglycolate medium.[4]

In a period of eight months, 250 burn wound samples were studied in patients who had second and third-degree burns. Two swabs were twirled simultaneously on a one centimeter square area. One swab was then smeared on a glass slide which was heat-fixed over a Bunsen burner and stained with Gram's technique.[5] The other swab was placed in the thioglycolate medium.

Methods of Isolation

Swabs were inoculated on MacConkey agar plates. After overnight incubation at 37°C, the plates were

Table I. Identification of 225 strains of Gram-negative bacilli isolated from burns.

ORGANISM	NO. OF STRAINS	PERCENTAGE
Pseudomonas aeruginosa	60	26.6%
Pseudomonas fluorescens	30	13.3%
Escherichia coli	36	16.0%
Enterobacter agglomerans	18	8.0%
Enterobacter cloacae	16	7.1%
Klebsiella pneumoniae	22	10.0%
Providencia stuartii	5	2.2%
Providencia retgeri	1	0.4%
Acinetobacter calcoaceticus Var. anitratus	12	5.3%
Acinetobacter calcoaceticus Var. lwoffi	5	2.2%
Proteus mirabilis	9	4.0%
Proteus morganii	2	0.9%
Proteus vulgaris	1	0.4%
Serratia marcescens	4	1.8%
Serratia liquifaciens	2	0.9%
Citrobacter freundii	2	0.9%

examined for Gram-negative bacteria. One colony of every growth form was picked for confirmatory tests and for morphological examination of a Gram-stained film.

Methods of identification

Gram-negative bacilli were identified with conventional methods and also for further confirmation with API-20 Profile Recognition System (Analytab Products Inc., Carle Place, N.Y.).

Antibiotic sensitivity tests

Strains were tested by the method of Bauer-Kirby.[6] A few colonies (three to ten) of the organism to be tested were lifted with a loop from the original culture plate and introduced into a test tube containing 4 ml of tripticase soy broth. These tubes were then incubated for two to five hours to produce a bacterial suspension of moderate cloudiness. The suspension was then diluted as necessary, with water or saline solution to a density visually equivalent to that of the standard prepared by adding 0.5 ml of 1% $BaCl_2$ to 99.5 ml of 1% H_2SO_4 (0.36 N).

The bacterial broth suspension was then streaked evenly in three planes onto the surface of Mueller-Hinton agar with a cotton swab (not a wire loop or glass rod).

Table II, A. Sensitivity to seven antibiotics by disk method of 225 strains of Gram-negative bacilli isolated from burns (May 1986 to Dec. 1986).

	B		CB		CL		CF		C		G		K	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R
P. aeruginosa	0	100	8.8	91.2	100	0	0	100	0	100	10	90	0	100
P. fluorescens	0	100	10	90	100	0	0	100	0	100	13	87	0	100
E. coli	0	100	33	67	35	65	36	64	37	63	66	34	0	100
E. agglomerans	0	100	0	100	81	19	0	100	37	63	99	1	0	100
E. cloacae	0	100	0	100	89	11	0	100	25	75	97	3	0	100
K. pneumoniae	0	100	0	100	100	0	100	0	73	27	99	1	0	100
P. stuartii	0	100	0	100	0	100	77	33	93	7	50	50	0	100
P. retgeri	0	100	0	100	0	100	79	31	94	6	54	46	0	100
A. anitratus	51	49	46	54	100	0	0	100	100	0	94	6	100	0
A. lwoffi	50	50	48	52	100	0	0	100	100	0	92	8	100	0
P. mirabilis	0	100	0	100	8	92	0	100	70	30	97	3	16	84
P. morganii	0	100	0	100	10	90	0	100	65	35	95	5	9	91
P. vulgaris	0	100	0	100	12	88	0	100	67	33	96	4	8	92
S. marsescens	0	100	13	87	10	90	0	100	69	31	100	0	77	33
S. liquifaciens	0	100	15	85	13	87	0	100	70	30	100	0	66	34
C. freundii	0	100	100	0	100	0	100	0	79	21	100	0	0	100

B = bacitracin, 10 units; CB = carbenicillin, 100 mcg; CL = colistin, 10 mcg;
CF = cephalothin, 30 mcg; C = chloramphenicol, 30 mcg; G = gentamicin, 10 mcg;
K = kanamycin, 30 mcg

Disks were placed on the agar with flamed forceps or a single disk applicator and gently pressed to ensure contact. Plates were incubated immediately for 30 minutes.

After overnight incubation, the zone diameters (including the 6mm disk) were measured with a ruler. The disks which we used in this study were from Biomerieux, Oxiod and Difco companies.

RESULTS

Table I shows the results of identification of 225 strains of Gram negative bacilli isolated from burns in two hospitals from May 1986 to December 1986.

Pseudomonas was the genus most frequently isolated, and the most common species was *Pseudomonas aeruginosa*. Next in frequency was *Escherichia coli*.

Table IIa shows the proportion of 225 of the strains listed in Table I which were sensitive and resistant to seven antibiotics by single disk method. The same data of six other antibiotics are shown in Table IIb.

A larger proportion of strains was sensitive to nalidixic acid, polymyxin B, gentamicin and colistin, and the lowest proportion of sensitive results was obtained with lincomycin and bacitracin.

Most of the strains, except pseudomonas spp. and proteus spp. were sensitive to a few antibiotics and caused no problem in relation to treatment.

Pseudomonas species were 100% sensitive to polymyxin B and colistin (polymyxin E), and 90% resistant to gentamicin and carbenicillin.

DISCUSSION

The proportions of different genera and species of aerobic Gram-negative bacilli isolated from burns were different from those normally found in the feces. While *E. coli* has a large preponderance in feces, the largest proportion of strains isolated from burns was pseudomonas species, of which *P. aeruginosa* was by far the most common species. In this respect, the bacteria of burns resemble those of secondary infection of the urinary tract, which include a large proportion of strains, including *P. aeruginosa* acquired from other patients or from environmental sources.[7]

Since *P. aeruginosa* is present in the feces of a relatively small proportion of normal individuals, the high incidence of these bacteria in burns not specifically protected against them must be attributed to infection from sources outside the patient.[8-10]

This view is supported by the presence in most cases of the infecting species in other patients before its first appearance in a burn wound.

The antibiotic sensitivity patterns of strains isolated from burns also support the view that a large proportion of these bacteria is acquired in hospital. Pseudomonas species showed 90% resistance to gentamicin and carbenicillin which was probably the result of the extensive use of gentamicin and carbenicillin in the treatment of patients in burn units. Generally however, nalidixic acid, gentamicin, colistin and polymyxin B were active against the largest number of strains.

Table II, B: Sensitivity to six antibiotics by disk method of 225 strains of Gram-negative bacilli isolated from burns (May 1986 to Dec. 1986).

	L		NA		N		PB		SXT		T	
	S	R	S	R	S	R	S	R	S	R	S	R
P. aeruginosa	0	100	0	100	0	100	100	0	0	100	4.4	95.6
P. fluorescens	0	100	0	100	0	100	100	0	0	100	6	94
E. coli	0	100	100	0	100	0	0	100	33	67	37	63
E. agglomerans	0	100	100	0	100	0	100	0	0	100	52	48
E. cloacae	0	100	100	0	100	0	100	0	0	100	57	43
K. pneumoniae	0	100	100	0	100	0	87	13	0	100	0	100
P. stuartii	0	100	100	0	0	100	0	100	0	100	0	100
P. retgeri	0	100	100	0	0	100	0	100	0	100	0	100
A. calcoaceticus anitratus	0	100	48	52	48	52	100	0	100	0	46	54
A. calcoaceticus lwoffi	0	100	51	49	49	51	100	0	100	0	48	52
P. mirabilis	0	100	92	8	0	100	83	17	16	84	0	100
P. morganii	0	100	93.5	6.5	0	100	83	17	18	82	0	100
P. vulgaris	0	100	93	7	0	100	81	19	16	84	0	100
S. marsescens	0	100	0	100	12	88	0	100	0	100	7	93
S. liquifaciens	0	100	0	100	17	83	0	100	0	100	5.5	94.5
C. freundii	0	100	100	0	100	0	100	0	100	0	100	0

L = lincomycin, 2mcg; NA = nalidixic acid, 30 mcg; N = nitrofurantoin, 300 mcg; PB = polymyxin B, 300 units; SXT = sulphamethoxazole-trimethoprim, 25 mcg; T = tetracycline, 30 mcg.

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