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Prevention of Gram-Negative Bacillary Pneumonia Using Aerosol Polymyxin as Prophylaxis. I. EFFECT ON THE COLONIZATION PATTERN OF THE UPPER RESPIRATORY TRACT OF SERIOUSLY ILL PATIENTS

Sheldon Greenfield, … , John Hedley-Whyte, David S. Feingold

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Research Article

A prospective study used polymyxin B by aerosol to reduce colonization of the upper respiratory tract with nosocomial gram-negative bacilli. 58 high-risk patients from the Respiratory-Surgical Intensive Care Unit entered the trial. 33 were randomly selected to receive 2.5 mg/kg/day of polymyxin B by hand atomizer into the pharynx, and tracheal tube if present. 17 of 25 control patients became colonized with gram-negative bacilli as compared with 7 of 33 polymyxin-treated patients ($p < 0.01$). Control patients became colonized with a total of 33 gram-negative bacilli: 3 were *Pseudomonas aeruginosa*, 21 were species of Enterobacteriaceae. The polymyxin-treated patients became colonized with a total of 11 gram-negative bacilli: no *P. aeruginosa* and only 3 species of Enterobacteriaceae were recovered. Colonization increased with duration in Respiratory-Surgical Intensive Care Unit and with time of required controlled ventilation. Polymyxin most effectively prevented the increase in colonization in treated patients who stayed in the Respiratory-Surgical Intensive Care Unit for longer than 1 wk and who required controlled ventilation for at least 72 h.

Prevention of Gram-Negative Bacillary Pneumonia Using Aerosol Polymyxin as Prophylaxis

I. EFFECT ON THE COLONIZATION PATTERN OF THE UPPER RESPIRATORY TRACT OF SERIOUSLY ILL PATIENTS

SHELDON GREENFIELD, DANIEL TERES, LEONARD S. BUSHNELL, JOHN HEDLEY-WHYTE, and DAVID S. FEINGOLD

From the Departments of Medicine and Anaesthesia of the Harvard Medical School, Beth Israel Hospital, and the Infectious Disease Unit, Beth Israel-Children's Hospital Medical Center, Boston, Massachusetts 02215

ABSTRACT A prospective study used polymyxin B by aerosol to reduce colonization of the upper respiratory tract with nosocomial gram-negative bacilli. 58 high-risk patients from the Respiratory-Surgical Intensive Care Unit entered the trial. 33 were randomly selected to receive 2.5 mg/kg/day of polymyxin B by hand atomizer into the pharynx, and tracheal tube if present. 17 of 25 control patients became colonized with gram-negative bacilli as compared with 7 of 33 polymyxin-treated patients ($p < 0.01$). Control patients became colonized with a total of 33 gram-negative bacilli: 3 were *Pseudomonas aeruginosa,* 21 were species of Enterobacteriaceae. The polymyxin-treated patients became colonized with a total of 11 gram-negative bacilli: no *P. aeruginosa* and only 3 species of Enterobacteriaceae were recovered. Colonization increased with duration in Respiratory-Surgical Intensive Care Unit and with time of required controlled ventilation. Polymyxin most effectively prevented the increase in colonization in treated patients who stayed in the Respiratory-Surgical Intensive Care Unit for longer than 1 wk and who required controlled ventilation for at least 72 h.

INTRODUCTION

Nosocomial respiratory infections are preceded by colonization of the upper respiratory tract (1). Thus, reduction in mortality from hospital-acquired gram-negative bacillary pneumonia may be achieved by preventing colonization with potential pathogens or by shifting the flora to less invasive organisms that colonize the upper respiratory tract.

A recent retrospective study (2) of pneumonia in our Respiratory-Surgical Intensive Care Unit (R-SICU) [1] described a high incidence of nonbacteremic *P. aeruginosa* pneumonia during the period 1967–1969, and documented the ineffectiveness of treatment with "appropriate" systemic antibiotics. Since the portal of entry is usually the upper respiratory tract, the feasibility of specific prophylaxis with localized antibiotic therapy was tested, and is reported in this study.

Polymyxin B was selected because of strong tissue binding (3), absence of easily acquired bacterial resistance (4), its broad range of effectiveness against gram-negative bacilli (GNB) and the evidence that polymyxin B aerosol prevented the implantation of *Pseudomonas* in the trachea of polio patients with tracheostomy (5). Since the upper respiratory tract is rapidly colonized after acute illness and hospitalization (1, 6) and since pneumonia often occurs before prolonged controlled ventilation (2), prevention must be attempted early in the course of a given critical illness.

In this prospective investigation, polymyxin B by aerosol was given to randomly selected patients within 24 h of admission to the R-SICU. The prophylactic antibiotic markedly reduced colonization of the upper respiratory tract, not only with *P. aeruginosa* but also

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[1] *Abbreviations used in this paper:* GNB, gram-negative bacilli; R-SICU, Respiratory-Surgical Intensive Care Unit.

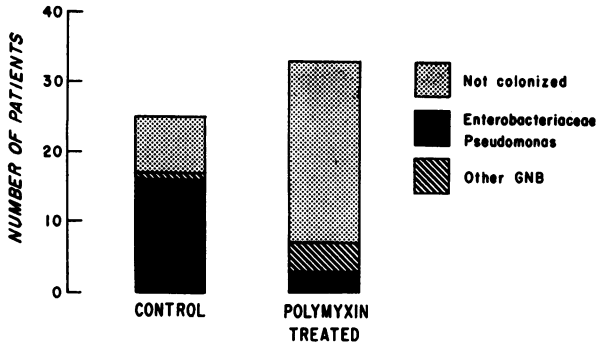

FIGURE 1 Predominating GNB causing colonization of the upper respiratory tract. Of 25 randomly selected control patients, 17 became colonized with GNB; 16 had species of Enterobacteriaceae or *P. aeruginosa*. Of 33 patients treated with 2.5 mg/kg/day polymyxin aerosol, only 7 became colonized with GNB ($P < 0.01$); only 3 of 7 predominating organisms were species of Enterobacteriaceae.

with polymyxin-sensitive organisms from pathogenic species of Enterobacteriaceae.

METHODS

The study was conducted in the R-SICU at the Beth Israel Hospital. Patients in respiratory distress from all services are admitted to this unit; the bulk of patients are those in whom respiratory difficulty is anticipated postoperatively. The cause and range of morbidity vary considerably. An extensive clinical description of the gram-negative bacillary pneumonias and bacteriology in the R-SICU had been recently completed (2). During the 2-yr period preceding the initiation of our study, 21% of the R-SICU patients developed gram-negative bacillary pneumonia while in the unit. The most common organism was *P. aeruginosa*, and the mortality rate of *Pseudomonas*-associated pneumonia was 71%.

Patients selected for inclusion into the study fulfilled the following criteria: estimated minimum stay of 72 h in the R-SICU (made at the time of admission), verbal consent of primary physician, informed written consent of the patient or legally responsible relative, random assignment to aerosol or control group, and initiation of aerosol treatment within 24 h of admission to the R-SICU. Neither respiratory failure, tracheostomy, nor intubation with an endotracheal tube was a requirement for inclusion. Criteria for exclusion were: preexisting pneumonia, prior colonization with *P. aeruginosa*, and significant renal failure (serum creatinine > 3.0 mg/100 ml at the time of admission to the R-SICU). Concurrent use of systemic antibiotics was not cause for exclusion.

Polymyxin B (Burroughs Wellcome Co., Research Triangle Park, N. C.), dissolved in saline solution, was administered by aerosol with a DeVilbiss hand atomizer (DeVilbiss Co., Medical Products Div., Somerset, Pa.) with a Rals power unit. A total of 2.5 mg/kg/day was given in six doses, one dose every 4 h. Approximately 6 ml of an 0.5% solution (5 mg/ml) was sprayed into the posterior pharynx. If the patient had a tracheostomy tube, one-half the dose was sprayed into the pharynx, and the other half into the tracheostomy tube. The second half-dose was delivered by syringe into the endotracheal tube if the patient was intubated. There was no attempt to deliver the aerosol into the distal bronchi.

Patients in the study were monitored as follows: chest X ray daily unless there was no clinical indication; complete blood count, blood urea nitrogen, and serum creatinine were measured twice weekly. The daily data collection for each patient was supervised by one of us (S. G. or D. T.) and appropriate clinical determinations were carried out as indicated, including alveolar-to-arterial oxygen tension difference at 100% inspired oxygen for 20 min ($AaDO_2^{1.0}$). Polymyxin levels in serum were determined in those patients with elevated serum creatinine. These were done by microbiologic assay with several strains of *Escherichia coli* (Sabath technique) (7).

Cultural methods and quantitation. Cultures of throat, and sputum if present, were taken daily. These cultures were usually collected in the morning after chest physiotherapy and within 1 h before the 10 a.m. polymyxin treatment. Swabs were planted directly on blood agar and MacConkey's agar. The swabbed segment was streaked with a loop in a standard way.

Cultures were graded in the following manner: + or few equaled 1–9 colonies on primary plating, ++ or moderate equaled 10–99 colonies, +++ or many equaled more than 100 colonies.

This semiquantitative approach was initially compared with a modification of the quantitative microbial culture method for sputum reported by Monroe, Muchmore, Felton, and Pirtle (8). Liquefaction was achieved by vortexing the specimen with 2% *N*-acetyl-L-cysteine; instead of calibrated loops, 0.1-ml aliquots of saline-diluted specimens were spread on chocolate, blood, and eosin-methylene blue agar plates. In general these direct comparisons showed that + correlated with $< 10^3$ organisms/ml, ++ with 10^3–10^4 organisms/ml, and +++ with 10^5 or more organisms/ml. The semiquantitative method was selected after these initial comparisons, since the time-consuming extra work involved in more precise quantitation did not appear to offer potential for acquiring additional important data. All cultures were speciated and interpreted by one bacteriologist.

Polymyxin B binds to tissue (3), and since we anticipated extensive dilution of the spray in 3 h, we felt that contamination of the cultures with the antibiotic would be negligible at 3 h after the spray. In four additional patients who were colonized with GNB sensitive to polymyxin B, throat cultures were taken at 5 min and then at 1 or 2 h after delivery of one dose of polymyxin aerosol into the posterior pharynx. These controls were designed to determine whether antibiotic contamination of the culture media was in fact negligible. GNB were identified by the criteria of Edwards and Ewing (9). Nonfermentative gram-

TABLE I

Age and Sex Distribution of the 58 Study Patients

	Control group		Polymyxin treatment group	
Age	Male/female	% in Age group	Male/female	% in Age group
20–39	2/1	12	3/2	15
40–59	3/3	24	4/4	24
≥60	7/9	64	11/9	61
Totals	12/13	100%	18/15	100%

negative bacilli were additionally identified as outlined by Gardner, Griffin, Swartz, and Kunz (10).

Definition of colonization. In this study colonization was defined by the isolation of a new organism from throat or sputum on more than one consecutive culture. If only a few colonies (nine or less on the primary plating) of GNB were present in the throat culture on admission to the unit, any increase on subsequent culture was considered to represent colonization.

Criteria for diagnosis of pneumonia. The diagnosis of pneumonia required the presence of a persistent alveolar infiltrate on at least two chest roentgenograms. Other causes for infiltrates such as pulmonary infarction and persistent atelectasis were excluded as best possible. This clinical judgment always included evaluation of the gram-stained sputum smear, the sputum culture, and temperature course of the patient, and the total and differential white blood count.

RESULTS

Between December 1970 and June 1972, 58 patients were admitted to the study. Of these, 25 were randomly selected as control patients and 33 were treated with polymyxin aerosol. 17 of the 25 control patients became colonized, compared with 7 of 33 prophylactically-treated patients ($P < 0.01$) (Fig. 1).

Since patients were not matched for variables, which might have produced differences in the incidence of colonization, the randomly selected patients were analyzed with respect to nine variables. The two groups were found to be similar for the following: age, sex, time in R-SICU, time on controlled ventilation, peak $AaDO_2^{1.0}$, fraction with GNB in the upper respiratory tract on admission to R-SICU, coincident morbidity, mortality, and concurrent systemic antibiotic treatment.

The age and sex distribution of the two groups is shown in Table I. Both groups had a similar fraction of young and old patients, and within each group the sex distribution was similar.

The control group spent a mean time of 7.6 days,

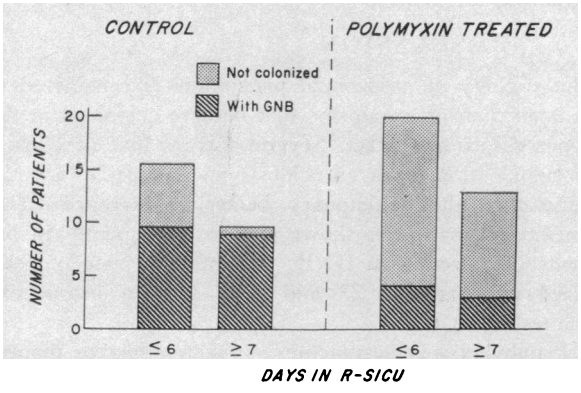

FIGURE 2 Effect of time on colonization. In control patients the longer the stay in the R-SICU the greater the incidence of colonization of the upper airway by GNB. Polymyxin aerosol prevented this colonization in 75% of treated patients, even after a week in the R-SICU.

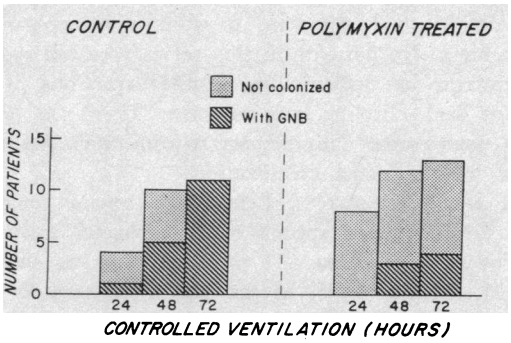

FIGURE 3 Colonization related to duration of controlled ventilation. Patients without a tracheal tube or those who received controlled ventilation for less than 24 h rarely become colonized with GNB. 50% of control patients who required 24–72 h of controlled ventilation became colonized with GNB. All 11 control patients became colonized after 72 h of controlled ventilation ($P < 0.02$). By contrast, polymyxin aerosol prevented colonization of two-thirds of patients who required at least 72 h of controlled ventilation.

with a median of 6 days, in the R-SICU. The polymyxin-treated group stayed an average of 9.0 days, but the median was also 6.0 days. Of 9 control patients in the R-SICU for 7 days or longer, 8 were colonized with GNB, whereas only 3 of 13 patients treated with polymyxin became colonized ($P < 0.05$, Fisher's exact test). Thus, polymyxin aerosol decreased the incidence of colonization by GNB in patients who spent 1 wk or longer in the R-SICU (Fig. 2).

The mean number of days in the study for control patients who required controlled ventilation was 4.2 days; the polymyxin-treated group was studied for a mean of 4.8 days. The median was 2.0 days in both groups. As can be seen in Fig. 3, 5 of 10 control patients who were ventilated for 48 h were colonized with GNB, while all 11 patients with prolonged ventilation were colonized ($P < 0.02$, Fisher's exact test). Polymyxin decreased colonization in patients undergoing prolonged controlled ventilation; 4 of 13 acquired GNB while all 11 control patients became colonized ($P < 0.01$, Fisher's exact test).

GNB were cultured from 8 of 25 control patients (32%) on admission to the R-SICU as compared with 10 of 33 polymyxin-treated patients (30%). While the patients were in the unit, there were 6 deaths among the 25 control patients and 4 deaths in the polymyxin group. The mean peak $AaDO_2^{1.0}$ in the control and polymyxin groups were 295 and 271 mm Hg, respectively. Of the 33 polymyxin-treated patients, 29 (88%) received parenteral antibiotics at some point during their stay in the R-SICU; 10 received ampicillin and/or cephalothin, 7 kanamycin, 3 chloramphenicol, and 2 gentamicin. Parenteral antibiotics were administered to 19 of 25 controls (76%); 9 received ampicillin and/or

cephalothin, 6 kanamycin, 6 chloramphenicol, and 1 gentamicin. No patients in this series received systemic polymyxins or carbenicillin. Similar fractions of both groups had chronic lung disease. Thus, the groups were comparable with respect to nine variables which could have affected colonization.

Of the 17 control patients who became colonized with GNB, 16 had species of Enterobacteriaceae (13) and/or *P. aeruginosa* (3) predominating, as shown in Fig. 1. Of the seven in the polymyxin-treated group who became colonized with GNB, three were colonized with species of Enterobacteriaceae. Two of these organisms were *Proteus* species which are naturally resistant to polymyxin B. No treated patient became colonized with *Pseudomonas*.

The 17 control patients became colonized with a total of 33 GNB, as shown in Table II; 24 were species of Enterobacteriaceae or *P. aeruginosa* and the remainder were other nonfermentative GNB. The 7 polymyxin-treated patients became colonized with 11 GNB; 2 patients acquired *Proteus* species and 4 had flavobacteria, that were also resistant to polymyxin. Five of the colonizing GNB were sensitive to polymyxin B including one Enterobacteriaceae.

Few pneumonias developed in these 58 patients during their stay in the R-SICU. Two patients in each group had pneumococcal pneumonia. Two control patients had nonbacteremic *Klebsiella pneumoniae*. However, in one of these patients the organism was present on admission to the R-SICU and the patient did not become colonized by our definition. Two patients, one in each group, had more than one GNB in the sputum and had fluctuating infiltrates on chest X ray but were not felt to have definite pneumonia.

GNB were cultured in 8 of 25 control patients on admission to the R-SICU. In two patients GNB did not persist. In three other patients the GNB increased; these patients became colonized by our definition. In the three remaining patients, the GNB did not change by the number of colonies cultured (not colonized) but these patients acquired other GNB.

TABLE II

Total Colonizing Organisms in the 17 Control Patients and 7 Polymyxin-Treated Patients who became Colonized with GNB

	Enteros.*	*P. aeruginosa*	*Proteus* and *Serratia*	Other nonfermentative GNB
Control group	15	3	6	9
Polymyxin treatment group	1	0	2	8‡

* Enterobacteriaceae excluding *Serratia* and *Proteus*.
‡ Primarily Herellea, and Flavobacteria.

TABLE III

Effect of Polymyxin Aerosol on the Enumeration of Polymyxin-Sensitive GNB in the Pharynx

Time of culture in relation to polymyxin aerosol	Patient 1	2	3	4
Before aerosol	*Pseudomonas* +*	*E. coli* +	*Klebsiella* +	*Klebsiella* + +
5 min after aerosol	*Pseudomonas* + +	*E. coli* +	*Klebsiella* +	*Klebsiella* + +
1 or 2 h after aerosol	*Pseudomonas* +	*E. coli* +	*Klebsiella* +	*Klebsiella* + +

* Direct platings from throat swabs to EMB agar were done by one person in the standard fashion employed throughout the study. + = 1–9 colonies; + + = 10–99 colonies; + + + = > 100 colonies of the specific bacterium.

GNB were cultured on admission from 10 of the 33 polymyxin-treated group. In five patients the GNB did not persist. In the other five, there was no increase in the quantity of GNB.

Our ability to culture existing polymyxin B-sensitive GNB from the throat after antibiotic aerosol in the four additional control patients who received one dose of polymyxin aerosol is shown in Table III. GNB were cultured successfully at 5 min and at 1 or 2 h after the spray with very little change in the number of organisms observed by our semiquantitative technique.

Six patients developed renal failure with elevated serum creatinine values ranging from 1.5 to 8.8 mg/100 ml. Polymyxin was detectable in the serum of only one of these patients at 1 μg/ml after the patient had received polymyxin for 5 days.[2]

Toxicity from the aerosol was not detected. There were no allergic reactions or episodes of acute respiratory distress. The nursing staff had no difficulty in preparing the solution.

DISCUSSION

The etiology of nosocomial pneumonia is considered to be aspiration of organisms that become colonized in the upper respiratory tract. Several factors that predispose to colonization, such as acidosis and azotemia, are the same that alter pulmonary bacterial clearance (11). Pneumonia has been shown to occur frequently in patients colonized with GNB; the infection rate of colonized patients was 23 and 25% in two prospective studies (1, 6).

Prophylaxis is best achieved when effective therapy is directed toward one or a few specific sensitive organisms (12). Previous attempts to sterilize the phar-

[2] The assay was performed by C. C. HsuChen, Infectious Disease Unit, Beth Israel Hospital. The microbiological assay can detect 0.5 μg/ml.

ynx and trachea have been unsuccessful. Lepper, Kofman, Blatt, Dowling, and Jackson (5) were unable to sterilize the tracheal flora in polio patients with tracheostomy with multiple antibiotic combinations. Johnston and Bodey (13) attempted to sterilize the oropharynx using vancomycin and neomycin. Gentamicin was recently administered prophylactically to neurosurgical patients with tracheostomy (14) but the aminoglycoside was given to the lower respiratory tract, and apparently resistant organisms emerged.

The aminoglycosides and polymyxins, administered by the parenteral route, have no appreciable effect on the upper respiratory tract flora, probably because adequate levels of antibiotic are not attained. Poor diffusion into tissue and toxicity limit the value of the polymyxins for the systemic treatment of gram-negative bacillary pneumonia. However, because of the absence of widespread and easily acquired resistance of most GNB to polymyxin (4), it appears to have promise when applied locally.

Proteus species and *Serratia marcescens* are naturally resistant to the polymyxins. In hospitals or intensive care units where *Proteus* and *Serratia* are prevalent this polymyxin regime would not be applicable. One possibility under these circumstances might be to use the aerosol in combination with a parenteral sulfonamide (15). This combination might be effective against these two species of organism because adequate levels of sulfonamide are reached in the upper respiratory tract (16).

The major hazard of prophylactic antibiotic treatment, the emergence of resistant organisms, was not encountered frequently in the treated patients. Only 6 GNB resistant to polymyxin were colonized in the 33 treated patients. The polio patients who received polymyxin aerosol in the study by Lepper et al. (5) became colonized with gram-positive organisms. Once colonization with *P. aeruginosa* has occurred, polymyxin is not very effective (13). Thus, the potential for selecting resistant organisms appears small.

The net effect of altering the bacterial flora within an intensive care unit from species of Enterobacteriaceae and *P. aeruginosa* to either gram-positive organisms which are more amenable to antibiotic therapy, or to nonfermentative GNB which may be less invasive, would likely be favorable (10, 17).

The long-term goal for the use of prophylactic polymyxin is to reduce the incidence of gram-negative bacillary pneumonias. The small number of cases in the control group precludes any conclusion about the ultimate prevention of pneumonia. At present, a further prospective study is in progress to determine if the incidence of gram-negative bacillary pneumonia in the R-SICU is reduced by prophylactic polymyxin aerosol treatment. We are also investigating whether any changes in the epidemiology of GNB occur within the R-SICU due to the widespread use of polymyxin B by aerosol. Since superinfection by nosocomial pathogens in patients hospitalized with community-acquired pneumonia is frequent and dangerous (6), successful prevention of colonization with GNB may be helpful for these patients also.

Toxicity from polymyxin was negligible. Polymyxin was not systemically absorbed since the aerosol was administered to the upper respiratory tract only, in contrast to therapeutic maneuvers where antibiotic by aerosol is delivered to lung parenchyma (18–20). The aerosol carrier, dichlorodifluoromethane, has been associated with cardiac arrhythmias in asthmatics (21). Since in our study the aerosol was not delivered to peripheral lung, systemic absorption was probably minimized. There have been several case reports of episodes of respiratory distress related to aerosol drug (22). None of these reactions were encountered in our patients.

A general factor which increases the probability of colonization with GNB is degree of illness (23). Because of indefiniteness of criteria, no attempt was made to grade degree of illness. Rather, several objective variables which reflect degree of illness were compared in the two groups and found to be similar: duration of controlled ventilation, peak $AaDO_2^{1.0}$, and duration of stay in the R-SICU.

Johanson, Pierce, and Sanford (23) found no correlation between acquisition of GNB and intermittent positive pressure breathing (IPPB) in moribund medical patients and moderately ill surgical patients. Duration of IPPB, however, was not tabulated. In their subsequent study (1), colonization with GNB was statistically correlated with tracheal intubation. The high colonization rate in R-SICU control patients who required controlled ventilation for more than 72 h may be explained by the degree of underlying illness rather than the apparatus used for ventilation (24).

Johanson, Pierce, Sanford, and Thomas (1) recently showed that colonization with GNB occurred during the first 4 days of hospitalization in patients admitted to a medical intensive care unit; rapid colonization with GNB also occurred in those patients with respiratory illness. The increase in colonization in control R-SICU patients who stayed more than 7 days, as compared with short-term admissions, may be explained by the fact that the R-SICU at Beth Israel Hospital is the referral area for critically ill patients with prolonged respiratory failure, including patients from the medical intensive care unit.

The dose and interval of administration of polymyxin were arbitrarily chosen to be similar to those of Lepper

et al. (5). A total daily dose of 2.5 mg/kg/day at an interval of 4 h may be more than required.

Growth inhibition of existing polymyxin-sensitive GNB in the flora by passive transfer of polymyxin B from the throat swabs to the culture media did not seem to be a problem in the patients in whom this was directly examined (Table III). In addition, our policy of taking cultures 3 h after the spray would seem to make important antibacterial contamination of the culture media even more remote.

We conclude that polymyxin, 2.5 mg/kg/day, given prophylactically by aerosol, significantly reduced the incidence of colonization of the upper respiratory tract by nosocomial GNB in postoperative patients and in patients with respiratory failure. Polymyxin effectively prevented the increase in colonization with GNB in treated patients who were in the R-SICU for 1 wk and who required prolonged controlled ventilation for 72 h or more. The method of administration could be performed routinely by the nurses.

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REFERENCES

1. Johanson, W. B., A. K. Pierce, J. P. Sanford, and G. D. Thomas. 1972. Nosocomial respiratory infections with gram-negative bacilli. The significance of colonization of the respiratory tract. *Ann. Intern. Med.* **77**: 701.
2. Stevens, R. M., D. Teres, J. J. Skillman, and D. S. Feingold. 1973. Pneumonia in an intensive care unit: a thirty month experience. *Arch. Intern. Med.* In press.
3. Kunin, C. M., and A. Bugg. 1971. Binding of polymyxin antibiotics to tissues: the major determinant of distribution and persistence in the body. *J. Infect. Dis.* **124**: 394.
4. Adler, J. L., and M. Finland. 1971. Susceptibility of recent isolates of *Pseudomonas aeruginosa* to gentamicin, polymyxin, and five penicillins, with observations in the pyocin and immunotypes of the strains. *Appl. Microbiol.* **22**: 870.
5. Lepper, M. H., S. Kofman, N. Blatt, H. F. Dowling, and G. G. Jackson. 1954. Effect of eight antibiotics used singly and in combination on the tracheal flora following tracheostomy in poliomyelitis. *Antibiot. Chemother.* **4**: 829.
6. Tillotson, J. R., and M. Finland. 1969. Bacterial colonization and clinical superinfection of the respiratory tract complicating antibiotic treatment of pneumonia. *J. Infect. Dis.* **119**: 597.
7. Saito, A., and L. D. Sabath. 1971. Rapid microassay of polymyxin B and colistin in blood. In the 11th Interscience Conference on Antimicrobial Agents, and Chemotherapy, 19–22 October, Atlantic City, N. J. 61. (Abstr.)
8. Monroe, P. W., H. G. Muchmore, F. G. Felton, and J. K. Pirtle. 1969. Quantitation of microorganisms in sputum. *Appl. Microbiol.* **18**: 214.
9. Edwards, P. R., and W. H. Ewing. 1962. Identification of Enterobacteriaceae. Burgess Publishing Company, Minneapolis. 2nd edition.
10. Gardner, P., W. B. Griffin, M. N. Swartz, and L. J. Kunz. 1970. Nonfermentative gram-negative bacilli of nosocomical interest. *Am. J. Med.* **48**: 735.
11. Kass, E. H., G. M. Green, and E. Goldstein. 1966. Mechanisms of antibacterial action in the respiratory system. *Bacteriol. Rev.* **30**: 488.
12. Leading Article. 1970. Prophylactic antibiotics. *Lancet.* **2**: 1231.
13. Johnston, D. A., and G. P. Bodey. 1972. Oropharyngeal cultures of patients in protected environment units. Evaluation of semiquantitative technique during antibiotic prophylaxis. *Appl. Microbiol.* **23**: 846.
14. Klastersky, J., G. Swings, and D. Daneau. 1972. Prevention of infections in tracheostomized patients with endothracheal gentamicin. In 12th Interscience Conference on Antimicrobial Agents, and Chemotherapy, 26–29 September, Atlantic City, N. J. 54. (Abstr.)
15. Greenfield, S., and D. S. Feingold. 1970. The synergistic action of the sulfonamides and the polymyxins against *Serratia marcescens*. *J. Infect. Dis.* **121**: 555.
16. Hoeprich, P. D. 1971. Prediction of antimeningococcic chemoprophylactic efficacy. *J. Infect. Dis.* **123**: 125.
17. Pedersen, M. M., E. Marso, and M. J. Pickett. 1970. Nonfermentative bacilli associated with man. III. Pathogenicity and antibiotic susceptibility. *Am. J. Clin. Pathol.* **54**: 178.
18. Ramirez-R, J., and E. F. O'Neill. 1970. Endobronchial polymyxin B. Experimental observations in chronic bronchitis. *Chest.* **58**: 352.
19. Rose, H. D., M. B. Pendharker, G. L. Snider, and R. C. Kory. 1970. Evaluation of sodium colistimethate aerosol in gram-negative infections of the respiratory tract. *J. Clin. Pharmacol.* **10**: 274.
20. Klastersky, J., C. Geuning, E. Mouawad, and D. Daneau. 1972. Endotracheal gentamicin in bronchial infections in patients with tracheostomy. *Chest.* **61**: 117.
21. Paterson, J. W., M. F. Sudlow, and S. R. Walker. 1971. Blood-levels of fluorinated hydrocarbons in asthmatic patients after inhalation of pressurised aerosols. *Lancet.* **2**: 565.
22. Marschke, G., and A. Sarauw. 1971. Danger of polymyxin B inhalation. *Ann. Intern. Med.* **74**: 296.
23. Johanson, W. G., A. K. Pierce, and J. P. Sanford. 1969. Changing pharyngeal bacterial flora of hospitalized patients. Emergence of gram-negative bacilli. *N. Engl. J. Med.* **281**: 1137.
24. Teres, D., P. Schweers, L. S. Bushnell, J. Hedley-Whyte, and D. S. Feingold. 1973. Sources of *Pseudomonas aeruginosa* infection in a respiratory/surgical intensive-therapy unit *Lancet.* **1**: 415.