AEROBIC GRAM-NEGATIVE PHARYNGEAL BACILLI OF ADULT ETHIOPIANS: CARRIAGE RATES AND ANTIBIOGRAMS

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Yohannes Mengistu

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ABSTRACT

The study was conducted to identify the types of Gram-negative pharyngeal bacilli and to determine their prevalence and antibiotic susceptibilities.

One-thousand pharyngeal swab specimens were processed: 300 from students, 303 from adult employees of Berhanena-Selam Printing Press (BSP), 200 from hospital staff and 197 from patients. The isolates were identified by standard biochemical tests. All isolates were tested for their sensitivities to 11 antibiotics using the Kirby-Bauer technique.

Forty-five (15%) students, 53 (17.5%) employees of BSP, 27(13.5%) hospital staff and 54(27.4%) patients were carriers of one or more Gram-negative bacilli (GNB).

The pharyngeal carriage rates of GNB were similar among the various groups of healthy subjects ($P>0.1$) but they were lower than that of the patients ($P<0.01$). The increase in prevalence was not correlated to antimicrobial therapy, but seems to be associated with underlying disease and duration of hospitalization. There was no association between isolation rates of GNB and age or sex.

Two hundred and nineteen strains of more than 18 species of Enterobacteriaceae or nonfermenting Gram-negative bacilli were isolated. Colony counts of these organisms were lower among healthy subjects than among patients. Klebsiella was

most frequently isolated (37%), followed by Pseudomonas (13.2%), Enterobacter (12.8%) and Acinetobacter (10%). Others were less frequently isolated. The frequency of isolation of Klebsiella was higher from students (49%) than from the other three study groups (32.5%; $P<0.05$).

The nonhospital isolates were more sensitive than the hospital isolates. Over 70% of the nonhospital isolates were sensitive to 8 of the 11 antibiotics but 55%, 32% and 30% were sensitive to cephalothin, carbenicillin and ampicillin respectively. Over 74% of the hospital isolates were sensitive to only polymyxin, gentamicin and trimethoprim-sulphamethoxazole. About 59-64% of the hospital isolates were sensitive to chloramphenicol, kanamycin and sulphadiazine, while to the other antibiotics only below 33% were sensitive. Only 4.4% of nonhospital isolates were sensitive to all. A few strains showed intermediate susceptibilities to antibiotics.

Multiple antibiotic resistance was higher among the hospital isolate (88.5%) than among the nonhospital isolates (59.6%; $P<0.01$). Eighty-three different resistance antibiograms of 1 to 10 antibiotics were detected. The resistance antibiograms were more varied among hospital isolates than among nonhospital isolates. Double resistance antibiograms were more frequent (28%) followed by triple resistance antibiograms (16%).

The findings were compared with reports from elsewhere. The high frequency of multiple antibiotic resistance of pharyngeal GNB seem to indicate the extensive use of antibiotics in the hospital and hence the associated problem of resistance to the antibiotics. A national policy for the rational use is strongly advised. The need for further research is stressed.

I. INTRODUCTION

Despite great advancement in the science of preventive medicine, microbial infection of man continues to be a major medical problem. Efforts have been made to study the microbiology of different areas of the body to understand what intrinsic and extrinsic factors allow the colonization of specific sites of the body by microorganisms and what make the organisms to initiate infection.

Respiratory tract infections are among the common infections of man (Neu, 1984). In Ethiopia, for example, respiratory tract infection is one of the main categories of disease and accounts for 17% of all hospital medical admissions (Editorial, 1974). Due to the increased use of antimicrobial agents and instrumentation in hospitals, antimicrobial resistant Enterobacteriaceae, Pseudomonas, Acinetobacter and Staphylococcus aureus strains are becoming the most frequent agents of opportunistic infections (von Graevenitz,1977). Among such infections, penumonia is a major cause of morbidity and mortality in hospitalized patients and to a less extent among community patients (Garb et al., 1978).

Many kinds of microorganisms colonize the upper respiratory tract including the throat. Because of the physical, chemical and biological factors, each body site may harbour

only a limited number of flora (Marsh, 1980; Mackowiak,1982). Given the appropriate circumstances, each member of the normal flora appears to have the capacity of helping or harming the host (Sprunt and Redman, 1968). The large number of potentially pathogenic microorganisms such as Gram-negative bacilli may in addition reflect infectious disease of the lower respiratory tract. Their detection in in the throat may also constitute useful guidance for clinical diagnosis (Lennette et al., 1980).

Normally, pharyngeal colonization with Gram-negative bacilli is thought to be less when compared to the other normal flora (Pierce et al., 1966; Rosenthal and Tager, 1975). Thus, their increase in prevalence may be associated with certain factors. This alteration may play an important part in the pathogenesis of Gram-negative bacillary pneumonia (Johanson et al., 1969;1972: Pierce and Sanford, 1974). Therefore, studying the prevalence of Gram-negative pharyngeal flora in normal healthy population and in patients may be important.

In Ethiopia, there has not been any study of the pharyngeal flora in general and Gram-negative bacilli of healthy subjects in particular (Messele Gedebou, Medical Faculty, Addis Ababa University, personal communication).

Laboratory experience with routine processing of throat swab specimens suggests low rate of isolation (Afeworki Gebre-Yohannes, National Health Research Institute, Addis Ababa, personal communication). Hence, it is important to study the pharyngeal flora of Ethiopians. The study was therefore initiated with the objectives to:

(1) determine the prevalence of aerobic Gram-negative bacilli colonizing the pharynx of healthy adult Ethiopians,

(2) identify the types of aerobic Gram-negative pharyngeal bacilli, and

(3) determine the antibiotic susceptibilities and obtain pertinent information on the antibiograms of the strains to be isolated.

The study comprised only aerobic GNB, because of their clinical significance particularly as frequent nosocomial pathogens.

The findings from such studies would be useful in the evaluation and interpretation of diagnostic bacteriological examination of sputum specimens. Although the results of this study may be considered applicable only to the selected population, it is believed that it may generally serve as a basis for better interpretation of reports of the throat specimen cultures.

II. LITERATURE REVIEW

1. The Throat as a Habitat

The throat is part of the upper respiratory tract and includes the laryngopharynx, the nasopharynx and the oropharynx. The oropharynx serves as a drainage channel for the passage of food and drink taken into the mouth. It is covered with pseudostratified ciliated columnar epithelium. The tonsils and the adenoid encircle the oropharynx and they are involved in the immune reactions (Ballenger, 1977).

The ecological properties of the throat enable the growth and colonization by limited number of bacterial flora. Bacteria which tolerate the factors that prevent the colonization are better adapted to this habitat. Such factors including gradients of pH, oxygen concentration, oxidation-reduction potential and nutrient availability will influence the oral flora (March,1980). Transient fluctuation in the stability of the oral ecosystem may be induced by the frequency and type of food ingested and the variation in saliva flow (Skinner and Carr, 1974).

2. Bacterial Throat Flora

Gram-positive bacteria: Gram-positive cocci are among the organisms most found in the various regions of the throat. These are micrococci (0-36%), Streptococcus pneumoniae (0-50%), Staphylococcus aureus (35-40%), Staphylococcus epidermidis

(30-60%), alpha and non-haemolytic streptococci (25-99%), and beta-haemolytic streptococci (15-20%) (Pelczar et al., 1977; Agarwal et al., 1981). Among the Gram-positive bacilli Corynebacterium species (50-90%) and Corynebacterium diphtheria (0-12%) may be found in the throat of healthy people.

Gram-negative bacteria: Neisseria species can be isolated in high numbers from most sites in the throat. Neisseria meningitidis is found in the mucus membranes of the oropharynx in 5-30% of healthy individuals in nonepidemic situations and in nearly 100% during epidemics (Devoe,1982). Haemophilus influenzae and Haemophilus parainfluenzae, the normal flora in the nasopharynx, could be found in the oropharynx of 5-30% of healthy people (Branson,1968;Chow et al.,1974).

Members of Enterobacteriaceae and glucose nonfermenting bacilli are less frequently isolated from healthy throat of man. It is not well understood whether they represent part of the "transient" or resident" pharyngeal flora (Rosenthal and Tager,1975; Irwin et al., 1982).

Members of the family Enterobacteriaceae are Gram-negative bacilli, nonsporeforming, facultative anaerobes, and oxidase negative. They attack sugars fermentatively, reduce nitrate to nitrite and can grow in ordinary media. The motile species possess peritrichous flagella (Lennette et al.,1980).

Infections with this family have become an increasingly important problem in medical practice. They represent important causes of community and hospital acquired infections. Some members of the family Enterobacteriaceae frequently colonize the upper respiratory tract of seriously ill patients and may thereby lead to hospital acquired pneumonia (Tillotson and Finland, 1969).

More than 80% of the glucose nonfermenting bacilli isolated from clinical specimens comprise Pseudomonas, Acinetobacter, and Moraxella (Pickett and Manclark,1970).

Pseudomonas species represent two-thirds of the nonfermenting bacilli recovered from clinical specimens. They are Gram-negative asporogenous bacilli,strictly aerobic, mostly motile with polar flagella and oxidase positive. They either fail to produce acid from carbohydrates or utilize these substrates oxidatively without the production of gas (Gilardi,1978). The most clinically important is Pseudomonas aeruginosa, which is an important cause of respiratory and other diseases.

Acinetobacter species are Gram-negative, strictly aerobic, nonmotile and oxidase negative bacilli. They may attack sugars oxidatively. Approximately one-third of the nonfastidious Gram-negative bacilli isolated in hospital laboratories are Acinetobacter species. Infections with these organisms usually follow medical instrumentation and antibiotic therapy (Gardner et al.,1970).

The genus Moraxella is Gram-negative bacillus, strict aerobe, nonmotile, oxidase positive and never attack carbohydrates (Gilardi,1978). Moraxella species are considered to be normal inhabitants of the upper respiratory tract and they may be isolated in the throat cultures with less frequency (Pedersen et al.,1970; Pickett and Pedersen 1970).

3. Mechanisms of Prevention of Pharyngeal Colonization with Gram-negative Bacilli

3.1 Host Defense Mechanism

Like other parts of the body the throat is prevented from infection by the powerful host defense mechanisms. There is an in situ mechanism for clearing bacteria in the upper respiratory tract. Mechanical cleansing forces are considered as potential factors influencing the bacterial flora of the oropharynx (Johanson,1984). The cleansing actions of the swallowing reflex, mastication and muscular movements of the tongue as well as the physical flow of saliva and other secretions across the surfaces are important means of preventing the colonization of potential pathogens (Kass et al., 1967).

According to LaForce et al., (1976) poorly defined bactericidal systems are believed to play a role in the regulation of the character and numbers of organisms that

make up the normal "throat flora". Lysozyme-like substances, lactoferrin and lactoperoxidase which are present in the human saliva may prevent the colonization of potentially pathogenic Gram-negative bacilli (Wilson and Miles, 1975). Secretory immunoglobulin A, which is the predominant class in secretions bathing the mucus membrane prevents adherence of bacteria to the epithelial surface (Williams and Gibbons, 1972; Pennington, 1984).

3.2 Inhibition of Gram-negative Bacilli by Normal Flora

The normal pharyngeal flora serve several functions; but of prime importance is their prevention or limitation of colonization by the pathogenic microorganisms such as Gram-negative bacilli (Sprunt and Redman, 1968; Mackowiak, 1982).

Studies on the role of normal flora in resistance to infection have shown the existence of numerous ways by which pathogens are prevented. Production of bacteriocins (high molecular weight protein antibiotics) by alpha-haemolytic (viridans) streptococci is considered to be an important barrier to colonization of the oropharynx by Streptococcus pyogenes (Sanders et al.,1976; Crowe et al., 1973), Streptococcus pneumoniae (Johanson et al.,1970), and Gram-negative bacilli (Sprunt and Redman,1968; Sprunt et al., 1971). There are probably other metabolic end products of

the normal flora that either are directly toxic to the new colonizers or inhibit the growth of these organisms indirectly by lowering local reduction-oxidation potentials (Sanders, 1969).

Normal flora may inhibit colonization of the potential pathogens by depleting essential nutrients and suppressing adherence. They may also enhance antibody and interferon production, and stimulate clearance mechanisms (Mackowiak, 1982).

4. Factors Associated with Gram-negative Bacilli Pharyngeal Colonization

Specific sites of human body like the pharynx allow the colonization of a limited number of microbial flora. However, due to various factors, this sites may be colonized by microorganisms which do not belong to the normal flora (von Graevenitz,1977).

Bacterial adherence to buccal epithelial cells is thought to be a mechanism facilitating oropharyngeal colonization and subsequent infection by the potentially pathogenic bacteria (Johanson et al.,1980). Different epithelial cells within anatomic sites as confined as the human pharynx have striking differences in suitability for adherence by individual bacterial species. Gibbons and van Houte (1971,1975) and Johanson et al. (1979) have shown that in human oropharynx,

bacteria with the strongest affinity for particular epithelial cell types *in vitro* are the same microorganisms colonizing the cell types *in vivo*.

Adhesins are microbial surface antigens that frequently exist as pili (Peachey,1981). Piliated strains, because of their enhanced adherence characteristics, have a selective advantage and ability to persist in the oral cavity (LaForce *et al.*, 1976).

General debilitation accompanying usually severe disease are potential sources of perturbation of the normal flora and the rise of potential pathogens. Johanson *et al.* (1969) and Rahal et al. (1970) have shown that the prevalence of Gram-negative bacilli is low in physiologically normal subjects, even if they are exposed to hospital environments. However, hospitalized patients, (Johanson *et al.*, 1969) elderly institutionalized patients, (Valenti *et al.*, 1978) chronic alcholics, and diabetics (Fuxench-Lopez and Rameriz-Ronda 1978; Mackowiak *et al.*, 1976,1978,1979) have been shown to have an abnormally high prevalence of Gram-negative bacilli in their pharynx. It has been hypothesized to be a result of "sick cells" that lack adequate clearance mechanisms (Welt,1967).

In addition, it is also presumed that the epithelial cells of ill persons favour the adherence of Gram-negative bacilli to a greater extent than do the epithelial cells of healthy individuals (Higuchi and Johanson,1980). Alterations in cell

surface protein (fibronectin) may mediate buccal cell adherence of bacilli. Fibronectin, a large molecular weight protein, is present on the surface of normal oropharyngeal epithelial cells (Yamada and Olden, 1978). This surface protein is highly sensitive to proteolytic enzymes such as trypsin (Yamada and Olden, 1978). Under severe illness or stress, protease activity increases and this results in the decrease of fibronection (Woods et al., 1981). If such a decrease of fibronectin persists, patients will be predisposed to develop Gram-negative bacillary pneumonia (Stevens et al.,1974).

Respiratory viruses, especially influenza viruses, are recently recognized group of exogenous agents which may promote pharyngeal colonization with Gram-negative bacilli. They facilitate the adherence of these bacteria to pharyngeal epithelial cells (Fainstein et al., 1980).

Tillotson and Finland (1969) have considered that age is an important factor, because the rate of colonization and superinfection with Gram-negative bacilli such as Pseudomonas, Enterobacter, Proteus, Klebsiella, Escherichia coli,Serratia and Acinetobacter occur in patients over 50 years old. This relationship to an altered immunologic status is as part of normal aging process or more to the acquisition of various chronic diseases with advancing age. This condition may predispose the individual to pulmonary infection (Tillotson and Lerner, 1966).

Sprunt and Redman (1968) have reported that antibiotic-induced suppression of the normal flora produces an ecologic vacuum that is rapidly filled by resistant organisms. This is thought to be a major factor in the aetiology of Gram-negative bacillary pneumonia (Pierce et al., 1966; Johanson et al., 1972; Bodey et al.,1983).

Dietary deficiency is thought to be at least partially responsible for the heightened susceptibility of the host. The carriage of different serotypes of Escherichia coli can be altered according to the type of diet (Bettelheim et al., 1977). There is an evidence that the meat of cattle, pigs and chickens (Shooter et al.,1970) and plant food, such as raw fruit vegetables and salad (Cooks et al.,1970) may alter the serotypes of E.coli. Enteric-Gram-negative bacilli occur commonly and in abundance in oropharyngeal secretions of some groups of malnourished children (Gracey et al., 1973; Gilman et al., 1982).

As an environmental factor high exposure to some potential pathogenic microorganisms may determine their prevalence. Studies made by Gracey et al., (1973;1979) have indicated that young children frequently harbour enteric-Gram-negative bacilli in their oropharynx. This was due to the high rate of exposure of these children to "fecal micro-organisms" in their crowded unsanitory living conditions.

5. Gram-negative Bacillary Pneumonia

Most cases of bacterial pneumonia are thought to be due to the microorganisms that inhabit the flora of the pharynx (Johanson et al.,1972). Two lines of evidence exist to support this concept. First, instillation of pneumonia into the nose of unanesthetized rabbits results in the appearance of the organisms in the lung within minutes. Second, occulsion of dog bronchi with sterile cotton plugs leading to atelectasis is associated with the recovery of pharyngeal organisms distant to the occulsion (Lansing and Jamieson,1963;Huxley et al.,1978).

The mechanism for the spread of pharyngeal bacterial flora to the lung remains unclear, but aspiration of oropharyngeal secretion has been suggested as the mechanism by which these bacteria reach lower respiratory tract (Pierce and Sanford, 1974; Huxley et al.,1978). Other minor contributing factors could be direct extension from continguous site of infection, aerosol inhalation and haematogenous spread from distant site of infection (Huxley et al.,1978).

Pneumonia occasionally develops in normal subjects, but it is more common in patients with conditions that depress host defense or enhance bacterial inoculation of the lung. Common situations such as drug and alcohol abuse, viral infection, cigarette smoking, age or haematogenic malignancy depress cellular defenses of the lung and may increase the risk of pneumonia (Palmer,1984). Of the patients with

community acquired pneumonia, 2-20% have Gram-negative bacilli as aetiologic agents, whereas, 45-60% nosocomial pneumonia are due to Gram-negative bacilli (LaForce,1981; Macfarlane et al., 1982).

Several clinical, microbiological and epidemiological studies have stressed the following general characteristics of Gram-negative bacillary pneumonia:

(1) Patients who are already seriously ill, whose respiratory tree is instrumented, who have undergone surgery (Glvor and Jolly, 1971; Schlenker et al., 1973) or patients receiving antimicrobial drugs are at greater risk for such infections (Redman and Lockey,1967; Glvor and Jolly,1971).

(2) Contamination from inhalation equipment has been clearly related to the outbreak of nosocomial respiratory tract infections (Dixton,1983).

(3) There is a significant relationship between pharyngeal colonization with Gram-negative bacilli and the development of pneumonia (Lefrock et al., 1979). Almost all aerobic Gram-negative bacilli are capable of causing these infections (Pennington et.al., 1973).

6. Diagnosis of Pneumonia

In pneumonia, signs and symptoms of respiratory infections are aetiologically nonspecific because noninfectious agents could also cause pulmonary infection (Palmer,1984). Thus the approach to diagnosis is largely dependent on clinical setting (Heineman and DiAntonio, 1982).

In hospital bacteriology laboratories, Gram stain and sputum culture are the most common techniques used for the diagnosis of pneumonia. Rarely does the sputum culture alone give accurate diagnosis in high risk patients because of the possible contamination by oropharyngeal organisms of potential pathogenicity (Bartlett, 1977; Mackowiak et al.,1978; Palmer et al., 1980). Thus, sputum culture has to be confirmed by positive blood cultures, visible morphologic change in the lung tissue, serologic detection of antigens or a specific increase of antibody (Whittle et al.,1974). Antimicrobial susceptibility testing of isolates (Heineman and DiAntonio, 1982) and serum antibacterial assay (Palmer,1984) are also helpful in guiding therapy.

In addition, the methods chosen must depend on local expertise in particular techniques, the location of the lung infiltrate (Peripheral versus central), the clinical condition of the patient, and the rapidity of progression of pneumonic process (Palmer, 1984). Among the diagnostic procedures to be followed are transtracheal aspiration (Bartlett et al.,1973; Bartlett, 1977; Brook, 1980). This permits access to lower respiratory tract that are devoid of oropharyngeal contamination. Transbronchial parenchymal biopsy open thoracotomy with lung biopsy (Greenman et al., 1975) and transthoracic needle aspiration of the lung (Bartlett et al.,1973; Palmer et al., 1980) are additional methods to be used.

III. MATERIALS AND METHODS

1. Study Groups

Four groups of subjects were studied in Addis Ababa from October, 1984 to March, 1985.

Group 1 were healthy students of Addis Ababa University. Their ages ranged from 17 to 25 years with a median of 19 and a mean of 19.5 years.

Group 2 were healthy adult employees of Berhanena-Selam Printing Press (BSP). The age range was 21-54 years with a median of 35 and a mean of 35.5 years.

Group 3 were hospital staff from Tikur Anbessa and Yekatit-12 hospitals. Their ages ranged from 20-53 years with a median of 27 and a mean of 29.5 years.

Group 4 were hospitalized patients from those two hospitals. The age range was 16-76 years with a median of 35 and a mean of 37 years.

2. Collection of Specimens

A standardized questionnaire was administered to each participant. Individuals among healthy subjects were excluded from the study if they had any of the following: sore throat, respiratory infection or any other chronic illness and use of antibiotics during the preceeding four weeks. For patients, clinical data were obtained from ward charts including days of hospital stay, diagnosis and antimicrobial therapy. Patients who had any type of respiratory disease were excluded from the study.

Specimens were obtained from the posterior part of the oropharynx with sterile cotton-tipped swabs moistened in 0.85% sterile saline solution. The tongue was depressed with sterile tongue depressor to prevent contamination.

3. Isolation and Biochemical Identification

The specimens were plated on MacConkey agar (Difco). The cultures were incubated for 24- 48 hours at 35-37°C before tests were discarded as negative. As a quality control, swabs were immersed in sterile saline solution (0.85%) and applied to the same media in an identical fashion.

A single colony was picked with a sterile straight wire and inoculated into 4 ml of nutrient broth (Difco). This was incubated at 35-37°C for 3-5 hours. From this young culture, motility was determined by hanging-drop preparation. Then the culture was used to inoculate tubes for biochemical tests.

The test and media employed include: Glucose,lactose and/or sucrose utilization (triple sugar iron agar,Oxoid), citrate utilization (Simmons' citrate agar,Oxoid),urease activity (urea agar,Oxoid), methyl-red and Voges-Proskauer test (Mr-VP medium, Oxoid), sulphide, indole production and motility (SIM medium,Oxoid) fermentation in 1% glucose,

mannitol, dulcitol inositol, sorbitol, salicin and adonitol (purple broth base, Difco), malonate utilization (malonate broth, Difco), oxidation-fermentation (OF) in 1% glucose, lactose and maltose (OF Basal medium, Difco) lysine iron agar (LIA, Oxoid) and oxidase reaction (oxidase disks, bio-Merieux). Indole production was tested with Kovac's reagent. Methyl red test was performed using methyl red solution, while, Voges-Proskauer test was performed using potassium hydroxide and alpha-naphthol solutions.

As quality control: (a) for sterility of media, uninoculated biochemical tubes were incubated at 35-37°C for 24-48 hrs, and (b) the ability of the media to show the desired reactions were determined by inoculating standard strains. All biochemical tubes under test were incubated at 35-37°C for 24-72 hours.

Members of the family Enterobacteriaceae were identified according to Edwards and Ewing (1972) and Cowan (1974), while for nonfermenting Gram-negative bacilli procedures according to Gilardi (1978) and Lennette et al. (1980) were used.

4. Antibiotic Susceptibility Testing

Eleven antibiotic disks (bioMerieux) were used. These are shown in Table 4. All isolates were tested for their susceptibilities to these antibiotics using the standardized agar disk diffusion technique (Bauer et al., 1966). The

inhibition zone diameters were interpreted as sensitive, intermediate or resistant according to Bauer et al.(1966).

As control, two standard reference strains were routinely tested. These were Escherichia coli (ATCC 25922) sensitive to all antibiotics used, and Pseudomonas aeruginosa (ATCC 27853) sensitive to carbenicillin, polymyxin B, and gentamicin.

5. Statistical Evaluation

The statistical significance of differences in isolations of Gram-negative bacilli and their rates of antibiotic resistance were assessed by means of X^2 (chi-square) or by normal deviate (Z-test) (Fleiss, 1973).

IV. RESULTS

1. Carrier Rates

One-thousand throat specimens were collected from the various groups: 300 specimens from university students, 303 from adult employees of Berhanena-Selam Printing Press (BSP), 200 specimens from hospital staff, and 197 specimens from hospitalized patients.

Carrier rates of Gram-negative bacilli among the various groups are shown in Table 1. Forty-five (15%) students, 53(17.5%) adult employees of BSP, 27(13.5%) hospital staff, and 54(27.4%) hospitalized patients were carriers of one or more Gram-negative bacilli. The carrier rates of the three groups of healthy students, adult employees of BSP and hospital staff were not significantly different from each other ($P>0.1$). The rate of colonization by Gram-negative bacilli (GNB) in patients (27.4%) was significantly higher than that in the healthy subjects (15.6%; $P<0.01$).

Table 2 shows the carrier rates of GNB between the two sexes. Out of 451 healthy males, 68(15.1%) were found to be carriers. Similarly 57(16.2%) of 352 females were found to be carriers. Carrier rates among the two sexes, or among those of the same sex in the different groups were similar. The highest rate observed was from female

Table 1

Carriage rates of Gram-negative bacilli among the different study groups.

Study group		Specimens No.	Positive Cultures No.	Positive Cultures Percent
NHAHS[1]	students	300	45	15.0
	adult employees of BSP[3]	303	53	17.5
	both groups	603	98	16.3
HAHS[2]	hospital staff	200	27	13.5
All healthy subjects		803	125	15.6
Hospitalized patients		197	54	27.4
Total		1000	179	17.9

1. NHAHS: Nonhospital associated healthy subjects.
2. HAHS: Hospital associated healthy subjects.
3. BSP: Berhanena-Selam Printing Press.

Table 2

Carriage rates of Gram-negative bacilli between males and females

Study Group	Male			Female		
	No. Specimens	Positive cultures		No. Specimens	Positive cultures	
		No.	%		No.	%
Students	196	31	15.8	104	14	13.5
Adult employees of BSP	182	27	14.8	121	26	21.5
Hospital staff	73	10	13.7	127	17	13.4
Hospitalized patients	118	33	28	79	21	26.6
All groups	569	101	17.8	431	78	18.1

employees of BSP(21.5%). This was not, however, significantly different (P>0.05) from the rates among the other groups. Out of 197 hospitalized patients, 118 were males and 79 were females. Twenty-eight percent males and 26.6% females were carriers of GNB.

As shown in Figure 1, healthy subjects whose ages were above 45 years had higher pharyngeal carriage rate of GNB (20.3%) than those whose ages were below 45 years old (15.2-15.3%). Hospitalized patients whose ages were below 30 years had lower carriage rate (22.6%) than that of patients whose ages were above 30 years old (31%; Figure 2). These differences in carriage rates were not significant (P>0.05.).

Among the patients, 94 had received antibiotics and 103 did not. Twenty-seven patients (28.7%) who had received antibiotics during the preceeding two weeks and the study period were carriers, while 27 patients (26.2%) who were not receiving any antibiotic were carriers of GNB. The administration of antibiotics had no significant effect on the prevalence of these organisms (P>0.1). Pharyngeal carriage rates among the patients was correlated to the duration of hospitalization. The longer the patients hospitalized the higher the colonization rate by GNB (Figure 3).

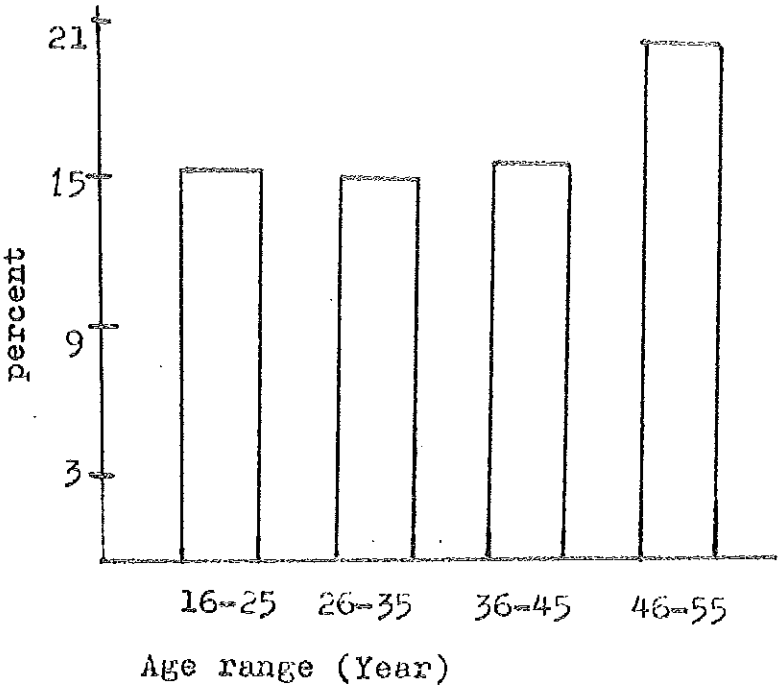

Figure 1. Percentage of positive pharyngeal cultures in relation to the age of healthy subjects

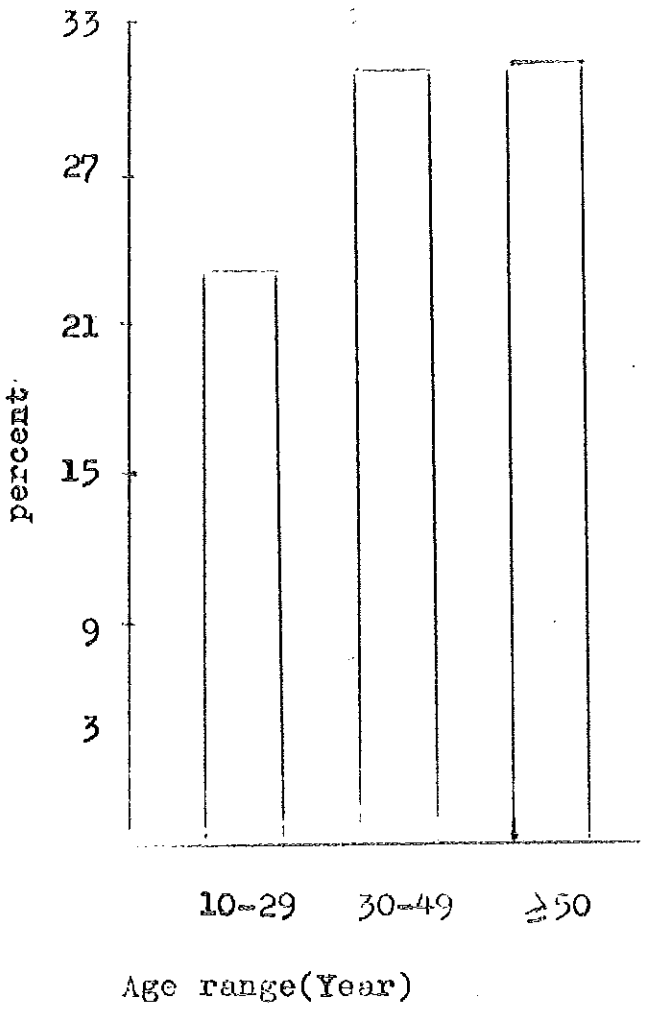

Figure 2. Percentage of positive pharyngeal cultures in relation to the age of patients

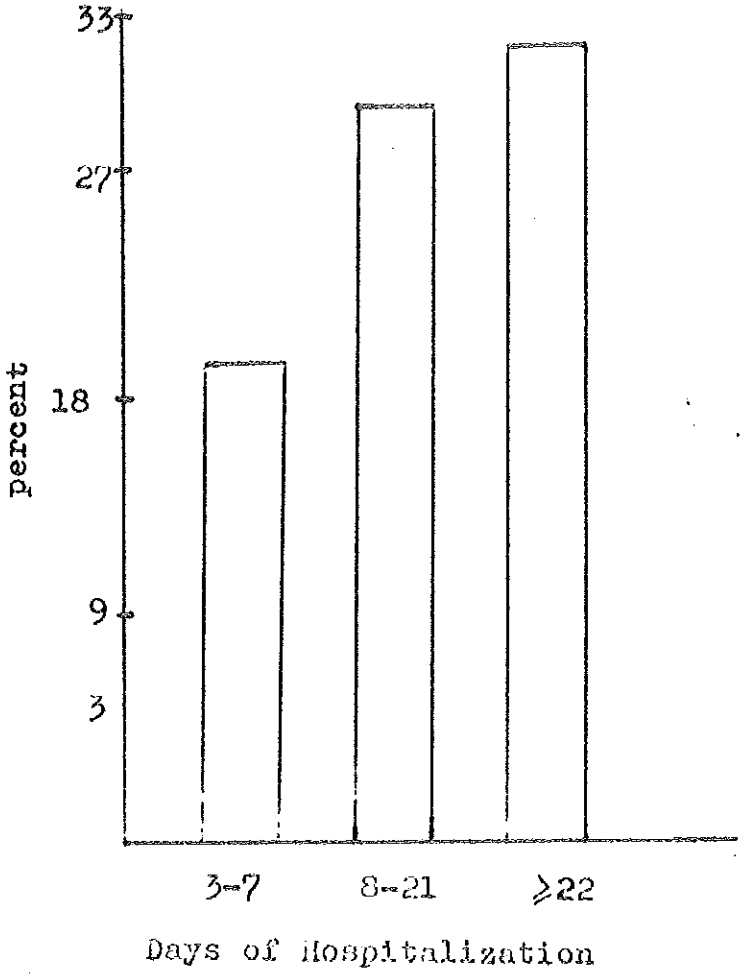

Figure 3. Percentage of positive pharyngeal cultures in relation to duration of hospitalization

2. Types and Frequencies of Bacterial Isolates

Two hundred and nineteen strains of aerobic Gram-negative bacilli were isolated from 179 positive pharyngeal cultures: 55(18.3%), 59(19.5%), 34(17%), and 71(36%) strains from students, adult employees of BSP, hospital staff and hospitalized patients respectively. From the various groups studied, more than 18 species were identified. These are shown in Table 3. Six different general of Enterobacteriaceae and four of nonfermenting Gram-negative bacilli were isolated in different frequencies.

There were more than 20 colonies isolated from the pharyngeal specimen of each patient in contrast to the much lower number of colonies from the similar specimen of each healthy individual. Usually, more than one type of species were not isolated from a single subject. Two types of species were isolated from 6(2%) adult employees of BSP, 10(3.3%) students, 7(3.5%) hospital staff and from 14(7.1%) hospitalized patients. Only 2(1%) patients had 3 species each.

As shown in Table 3, there were some species which were not isolated in all the groups studied. The two species of Proteus (P. mirabilis and P.rettgeri) were isolated from patients only, whereas, P.vulgaris and Alcaligenes faecalis were isolated from hospital staff

Table 3

Gram-negative bacilli isolated from pharyngeal cultures among the different study groups.

Bacteria isolated	Study group			
	Students (300)*	Adult employees of BSP (303)	Hospital staff (200)	Hospitalized patients (197)
	No.(%)**	No (%)	No(%)	No(%)
Klebsiella pneumoniae	17 (5.7)	12 (4)	5 (2.5)	19(9.6)
Klebsiella ozaenae	10 (5.7)	7 (2.3)	6 (3)	2 (2.5)
Enterobacter aerogenes	2 (0.7)	5 (1.6)	1 (0.5)	2 (1)
Enterobacter hafniae	1(0.3)	1 (0.3)	1 (0.5)	2 (1)
Enterobacter liquefaciens	1 (0.3)	-	-	5 (2.5)
Enterobacter cloacae	2 (0.7)	1 (0.3)	2 (1)	2 (1)
Escherichia coli	3 (1)	5 (1.6)	4 (2)	8 (4.1)
Citrobacter freundii	4 (1.3)	-	1 (0.5)	4 (2)
Proteus vulgaris	-	2 (0.7)	2 (1)	-
Proteus mirabilis	-	-	-	2 (1)

Table 3 (continued)

Bacteria isolated	Study groups			
	Students (300)*	Adult employees of BSP (303)	Hospital staff (200)	Hospitalized patients (197)
	No. (%)**	No (%)	No(%)	No(%)
Proteus morganii	2 (0.7)	2 (0.7)	-	1 (0.5)
Proteus rettgeri	-	-	-	1 (0.5)
Serratia marcescens	-	1 (0.3)	2 (1)	2 (1)
Pseudomonas aeruginosa	3 (1)	5 (1.6)	1 (0.5)	5 (2.5)
Pseudomonas spp	2 (0.7)	4 (1.3)	2 (1)	7 (3.6)
Acinetobacter spp	6(2)	9 (3)	2 (1)	5 (2.5)
Moraxella spp	-	-	3 (1.5)	-
Alcaligenes faecalis	-	1 (0.3)	2 (1)	-
Unidentified GNB	1 (0.3)	4 (1.3)	-	1 (0.5)
Total	55 (18.3)	59 (19.5)	34 (17)	71 (36)

* Figures in parentheses indicate total number of specimens

** percentage is out of the total number of specimens of each study group.

\- not isolated

and adult employees of BSP. One and five strains of Enterobacter liquefaciens were isolated from the student and patient groups respectively. Three strains of Moraxella species were isolated from hospital staff only. Six strains of nonfermenting bacilli which were not identified by the available technique were grouped under "unidentified GNB". They were isolated from the three groups: 4 strains from adult employees of BSP, and one strain each from student and patient groups.

Figure 4 shows the frequency of isolation of each genus out of the total isolates. Of all the Gram-negative pharyngeal isolates, the genus Klebsiella was most frequently isolated. It accounted for 37% of the total isolates. The second most frequent isolate was genus Pseudomonas (13.2%) followed by Enterobacter (12.8%) and Acinetobacter (10%). The rest of the isolates were less frequently isolated (10%).

The rate of Klebsiella among the three groups of isolates (staff, patient and adult employees of BSP isolates) were similar and accounted for 32- 34% of the isolates. But these were lower than 49% of the student isolates of Klebsiella ($P<0.05$).

3. Susceptibility to Antibiotics

All the strains isolated from the various groups of subjects were tested for their susceptibility to 11 antibiotics

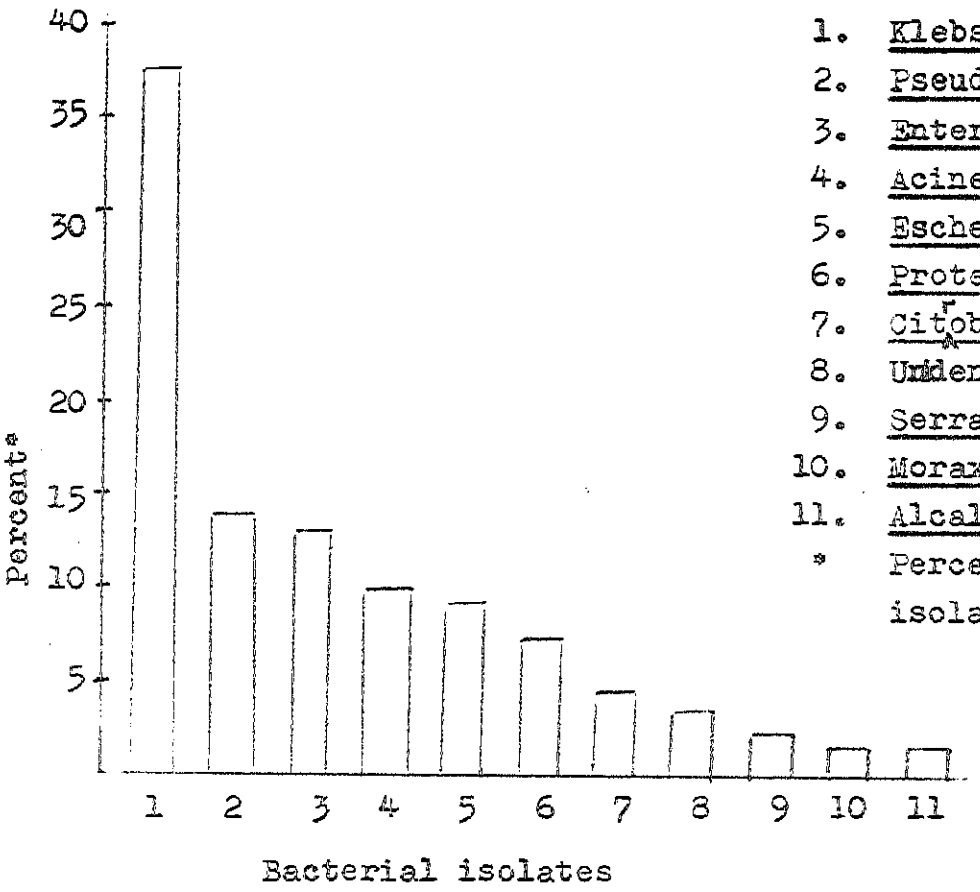

Figure 4. Frequency of Gram-negative bacilli isolated from pharyngeal cultures.

(Table 4). As shown in Table 5, over 92% of the isolates were sensitive to gentamicin and polymyxin B. Ninety-one percent of the nonhospital strains (isolates from non-hospital associated healthy subjects) and 63.8% of the hospital strains (isolates from both hospital staff and patients) were sensitive to kanamycin. Ninety-four percent of nonhospital strains and 74.3% of hospital strains were sensitive to trimethoprim-sulphamethoxazole. Ninety-three percent of nonhospital strain, 70.6% of staff strains and 56.3% of patient strains were found to be sensitive to sulphadiazine.

Streptomycin was effective against 70.2% of the non-hospital strains, 56% of staff strains and 34% of patient strains. Tetracycline was effective against 75.4%, 47% and 28% of nonhospital, staff and patient strains respectively. Sixty-three percent of hospital strains and over 80% of nonhospital strains were sensitive to chloramphenicol.

Ampicillin, carbenicillin and cephalothin were the least effective antibiotics against most of the pharyngeal isolates. Thirty-four percent of hospital strains and 55.3% of nonhospital strains were sensitive to cephalothin. Between 22% and 33% of isolates were sensitive to carbenicillin. Ampicillin was effective against 29.8% and 11.4% of non-hospital and hospital strains respectively.

Table 4

Antibiotics used in susceptibility tests of Gram-negative pharyngeal isolates.

Antibiotic	Abbreviation	Disk potency
Ampicillin	Amp	10mcg
Carbenicillin	Car	50mcg
Cephalothin	Cep	30mcg
Chloramphenicol	Chl	30mcg
Gentamicin	Gen	10mcg
Kanamycin	Kan	30mcg
Polymyxin B	Pol	300 units
Streptomycin	Str	10mcg
Sulphadiazine	Sul	1mg
Tetracycline	Tet	30mcg
Trimethoprim-sulphamethoxazole	Sxt	25mcg

Table 5

Sensitivities of nonhospital and hospital strains of Gram-negative bacilli to antibiotics .

Sensitive to	Nonhospital strains(114)		Hospital strains(105)		Total (219)	
	No.	percent	No.	percent	No	percent
ampicillin	34	29.8	12	11.4	46	21.0
carbenicillin	37	32.5	24	22.9	61	27.9
cephalothin	63	55.3	36	34.3	99	45.2
chloramphenicol	92	80.7	66	62.9	158	72.1
gentamicin	113	99.1	103	98.1	216	98.6
kanamycin	104	91.2	67	63.8	171	78.1
polymyxin B	106	93.0	97	92.4	203	92.7
streptomycin	80	70.2	45	42.9	125	57.1
sulphadiazine	106	93.0	62	59.0	168	76.7
tetracycline	86	75.4	34	32.4	120	54.8
trimethoprim-sulphamethoxazole	107	93.9	78	74.3	185	84.5
all antibiotics	5	4.4	0	0	5	2.3

Only 4.4% nonhospital strains were sensitive to all antibiotics. These include 3 strains of Klebsiella, one strain of Serratia marcescens and one unidentified strain. No single hospital isolate was found to be sensitive to all antibiotics.

Table 6 shows the frequency of strains with intermediate susceptibilities. It was less than 20% of the total isolates that were of intermediate susceptibility to any of the antibiotics used. Intermediate suscepbibilities to antibiotics was higher among the nonhospital isolates than the hospital isolates. No isolate with intermediate reading of susceptibility to polymyxin B or gentamicin was detected, except one strain of Klebsiella with polymxin B.

As shown in Figure 5, the hospital isolates showed higher resistance rates to any of the antibiotics than the nonhospital isolates. Resistance of the Gram-negative pharyngeal isolates to one or more antibiotics is shown in Table 7. Resistance to one antibiotic only was less than 14% of the total isolates. Resistance to one or more antibiotics was detected in 96.2% and 78.9% of hospital and nonhospital isolates respectively.

Ninety-seven percent and 71.7% hospital and nonhospital Klebsiella strains were resistant to one or more antibiotics. Strains of Escherichia coli isolated from the various groups

Table 6

Intermediate susceptibilities of Nonhospital and Hospital strains of Gram-negative bacilli to antibiotics

Intermediate to	Nonhospital strains(114)		Hospital strains(105)		Total(219)	
	No.	percent	No.	percent	No	percent
ampicillin	11	9.6	5	4.8	16	7.3
carbenicillin	27	23.7	13	12.4	40	18.3
cephalothin	4	3.5	8	7.8	12	5.5
chloramphenicol	8	7.0	9	8.6	17	7.8
gentamicin	-	-	-	-	-	-
kanamycin	7	6.0	9	8.6	16	7.3
polymyxin B	-	-	1	0.96	1	0.5
streptomycin	20	17.5	20	19.0	40	18.3
sulphadiazine	1	0.9	12	11.4	13	5.9
tetracycline	23	20.0	21	20.0	44	20
trimethoprim-sulphamethoxazole	3	2.6	2	1.9	5	2.3

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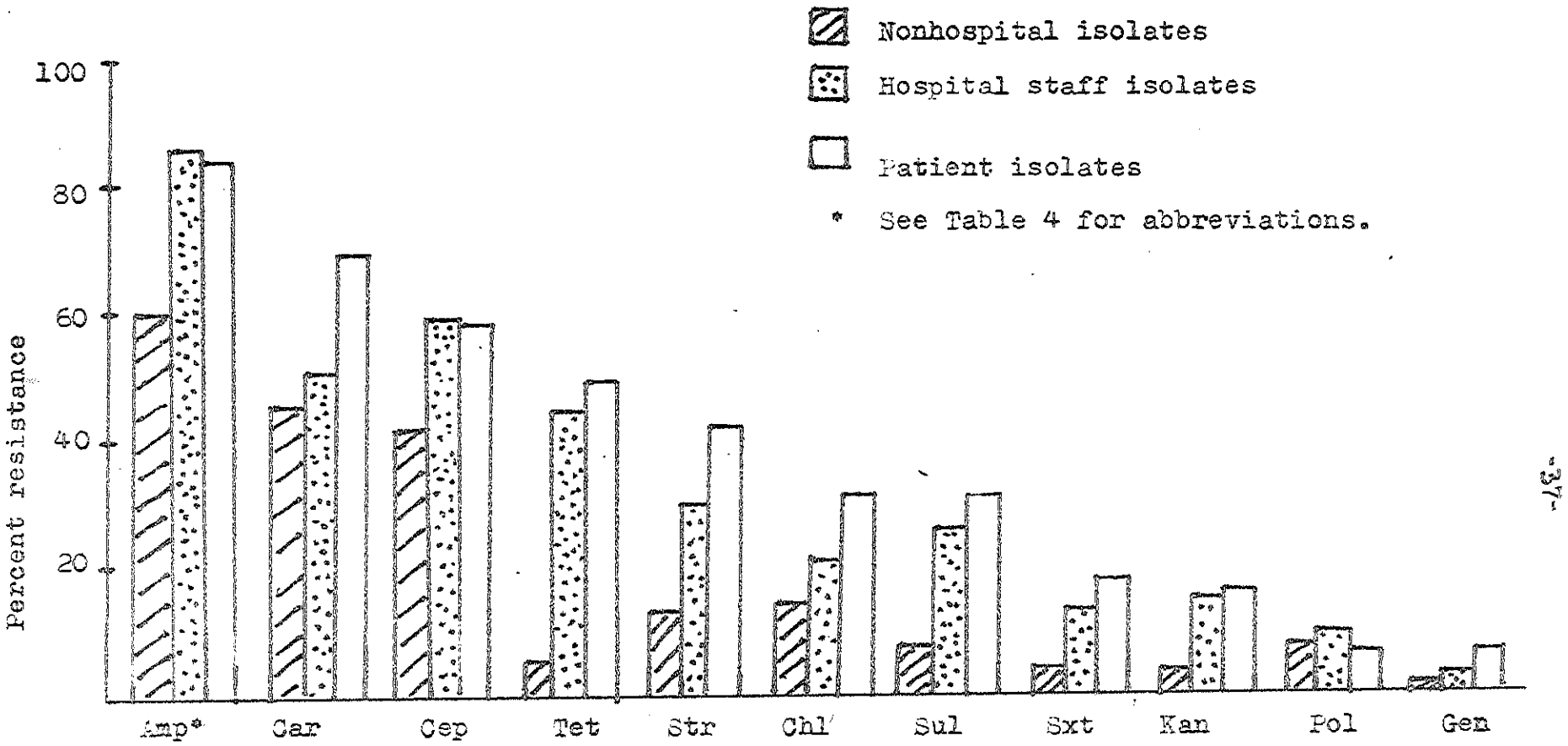

Figure 5. Resistance rates of isolates from various groups to individual antibiotics

Table 7

Single and multiple antibiotic resistance of nonhospital and hospital strains of Gram-negative bacilli.

Resistant to:	Nonhospital strains(114)		Hospital strains(105)		Total (219)	
	No.	percent	No.	percent	No	percent
one	22	19.3	7	6.6	29	13.2
two	35	30.7	26	24.8	61	27.8
three	18	15.8	17	16.2	35	16.0
four	6	5.3	15	14.3	21	9.6
five	3	2.6	10	9.5	13	5.9
six	5	4.4	10	9.5	15	6.8
seven	0	0	5	4.8	5	2.3
eight	0	0	5	4.8	5	2.3
nine	1	0.9	3	2.8	4	1.8
ten	0	0	3	2.8	3	1.3
one or more	90	78.9	101	96.2	191	87.2
two or more	68	59.6	94	89.5	162	74.0

showed variability in resistance to each of the antibiotics. All E. coli patient strains, 50% of the hospital staff and 37.5% of nonhospital strains were resistant to one or more antibiotics.

Multiple antibiotic resistance (resistance to two or more antibiotics) among the nonhospital isolates (59.6%) was significantly lower than the 89.5% of the hospital isolates ($P<0.05$) (Table 7).

A wide variety of resistance patterns (antibiograms) was observed among the different Gram-negative pharyngeal isolates. The resistance antibiograms are shown in Table 8. Eighty-three different resistance antibiograms were observed among 191 isolates. The hospital isolates showed more varied types of resistance antibiograms (77 types) than the nonhospital isolates (42 types).

In almost all the more frequent resistance antibiograms there was also resistant to ampicillin, carbenicillin and/or cephalothin. Single resistance antibiogram was observed in 29(13.2%) of strains. The most frequent was resistance to cephalothin demonstrated with 8 out of 22 strains of Acinetobacter.

Double resistance antibiograms were detected in 61(28%) strains of the total isolates. The commonest was ampicillin-carbenicillin combination. Among the strains that showed such resistance, 38(46.9%) were strains of Klebsiella

followed by 4(14.2%) strain of Enterobacter. Triple resistance antibiogram was similar in both hospital and nonhospital isolates (34 strains, 15.5%). The commonest pattern was ampicillin-cephalothin-polymyxin and it was observed in 8(54%) strains of Proteus.

The resistance antibiograms to four antibiotics were accounted by 21(9.6%) strains of the total isolates. Resistance antibiograms to five antibiotics was higher among hospital isolates (9.5%) than nonhospital isolates (2.6%). Five nonhospital strains (4.4%) and 10 hospital strains (9.5%) showed resistance antibiograms to six antibiotics.

No resistance antibiogram to seven, eight or nine antibiotics was observed among the nonhospital isolates, except in one strain of Pseudomonas which was resistant to nine antibiotics. Six (5.7%) hospital isolates were resistant to seven antibiotics.

Only 5(7%) patient strains showed resistance antibiograms to 8 antibiotics. Resistance antibiogram to 9 or 10 antibiotics was observed only in 3 strains of Pseudomonas.

Table 8

Resistance antibiograms of pharyngeal isolates of Gram-negative bacilli

	Resistance Antibiograms	No.	%
	Amp	3	3.7
	Car	2	2.5
Klebsiella	Amp Car	38	47
(81)	Amp Cep	3	3.7
	Amp Tet	1	1.2
	Amp Car Cep	2	2.5
	Amp Car Str	1	1.2
	Amp Car Tet	2	2.5
	Amp Cep Tet	1	1.2
	Amp Car Cep Pol	1	1.2
	Amp Car Str Sul	2	2.5
	Amp Car Cep Str Tet	2	2.5
	Amp Car Chl Str Sul	1	1.2
	Amp Car Chl Str Tet	1	1.2
	Amp Car Str Sul Tet	1	1.2
	Amp Car Chl Str Sul Tet	2	2.5
	Amp Car Chl Str Sul Sxt	1	1.2
	Amp Car Chl Kan Str Sul Tet	2	2.5

Table 8 (continued)

	Resistance Antibiograms	No.	%
	Amp	4	5.3
Other*	Cep	8	10.7
	Chl	1	1.3
Enterobacteriaceae	Sul	1	1.3
(75)	Amp Car	4	5.3
	Amp Cep	6	8.0
	Amp Tet	1	1.3
	Cep Tet	2	2.7
	Pol Tet	1	1.3
	Amp Car Cep	4	5.3
	Amp Car Tet	3	4.0
	Amp Cep Pol	7	9.3
	Amp Cep Tet	3	4.0
	Amp Car Cep Chl	1	1.3
	Amp Car Cep Tet	1	1.3
	Amp Car Pol Tet	1	1.3
	Amp Car Str Tet	1	1.3
	Amp Cep Pol Str	1	1.3
	Amp Cep Pol Sul	1	1.3
	Amp Cep Str Tet	1	1.3
	Amp Cep Sul Tet	1	1.3
	Car Kan Str Tet	1	1.3

Table 8 (continued)

Resistance antibiograms of Pharyngeal isolates of Gram-negative bacilli

	Resistance antibiograms	No.	%
Other* Enterobacteriaceae (75)	Amp Car Cep Pol Sul	1	1.3
	Amp Car Cep Pol Tet	1	1.3
	Amp Car Cep Str Tet	2	2.7
	Amp Car Cep Chl Str Tet	2	2.7
	Amp Car Cep Str Sul Tet	1	1.3
	Amp Cep Str Sul Tet Sxt	1	1.3
	Amp Car Cep Str Sul Tet Sxt	1	1.3
	Amp Car Chl Kan Str Sul Tet	1	1.3
	Amp Car Cep Pol Str Sul Tet Sxt	1	1.3
Gram-negative nonfermenting bacilli** (63)	Cep	10	15.9
	Amp Car	2	3.2
	Amp Cep	1	1.6
	Car Sup	1	1.6
	Cep Str	1	1.6
	Amp Car Cep	3	4.8
	Amp Car Str	1	1.6
	Amp Cep Chl	3	4.8
	Amp Cep Str	3	4.8
	Cep Chl Str	1	1.6
	Cep Sul Sxt	1	1.6

Table 8 (continued)

Resistance Antibiograms	No.	%
Amp Car Cep Chl	2	3.2
Amp Car Cep Str	1	1.6
Amp Car Cep Sul	1	1.6
Amp Car Cep Tel	2	3.2
Amp Car Cep Sxt	1	1.6
Amp Cep Chl Tet	1	1.6
Amp Cep Str Tet	1	1.6
Amp Chl Str Tet	1	1.6
Amp Car Cep Chl Sul	1	1.6
Amp Car Cep Chl Sxt	1	1.6
Amp Car Cep Str Sul	1	1.6
Amp Car Chl Sul Tet	1	1.6
Amp Car Cep Chl Kan Str	3	4.8
Amp Car Cep Chl Str Tet	1	1.6
Amp Car Cep Chl Tet Sxt	2	3.2
Amp Cep Chl Kan Str Sul	1	1.6

Table 8 (continued)

Resistance antibiograms	No.	%
Amp Cep Chl Kan Sul Tet	1	1.6
Amp Car Cep Kan Str Sul Sxt	1	1.6
Amp Car Cep Chl Str Sul Tet Sxt	1	1.6
Amp Cep Chl Kan Str Sul Tet Sxt	3	4.8
Amp Car Cep Chl Gen Kan Str Sul Sxt	3	4.8
Amp Car Cep Chl Gen Kan Str Tet Sxt	1	1.6
Amp Car Cep Chl Gen Kan Str Sul Tet Sxt	3	4.8

* Includes Enterobacter (28 strains), E.coli (20 strains), Proteus (13 strains), Citrobacter (9 strains) and Serratia (5 strains)

** Includes Pseudomonas (29 strains), Acinetobacter (22 strains) Moraxella (3 strains), Alcaligenes (3 strains), and unidentified GNB (6 strains).

V. DISCUSSION

The pharyngeal carriage rates of Gram-negative bacilli (GNB) were similar among the various occupational groups of healthy subjects studied. Healthy hospital staff and healthy nonhospital subjects have similar carriage rates as in the findings of Johanson et al. (1969) and Rahal et el. (1970).

There are no many reports on pharyngeal isolates of GNB from elsewhere. The pharyngeal isolation rate of GNB among healthy subjects (15.6%) in this study as a whole is similar to 14% and 18% reported respectively from Puerto Rico (Fuxench-Lopez and Ramirez-Ronda, 1978) and USA (Rosenthal and Tager,1975; Mackowiak et al.,1978). The isolation rate in this study is higher than the 12%,11.5%, 9%,6% and 2% of the normal control groups reported respectively by Mackowiak et al. (1976; U.S.A.), Rahal et al. (1970,U.S.A.), Philpot et al. (1980; Australia), Hable et al. (1971;U.S.A.) and Johanson et al.(1969; U.S.A.). On the other hand it is lower than 24% or 36% reported by Mackowiak et al (1979) in U.S.A. or Philpot et al. (1980) in Malaysia.

Differences in the isolation rates have been attributed to various factors such as climatic factors, food preference or other social habits (Philpot et al.1980). In contrast to Philpot et al.(1980), Gilman et al.(1982) suggested that living in tropics does not, by itself, appear to predispose an

invididual to GNB colonization. Bettelheim et al.(1977) have shown that diet can influence the carriage rate of different serotypes of Escherichia coli. In addition, different sampling and culture methods may explain the varying prevalence rates in the different studies. Rosenthal and Tager (1975) have recommended the use of selective enrichment broth in addition to the common culture media. This was not adopted in the present study. For economical reason or another, it may not be reasonable to use such media for routine diagnostic purposes.

In this study, the increase in prevalence of colonization of the pharynx of patients by GNB has not been associated with antibiotic treatment as in the findings of Johanson et al.(1969), LeFrock et al.(1979) and Irwin et al.(1982). This contrasts with the findings of Tillotson and Finland (1969) and Pollack et al.(1972). In addition, the finding in this study indicates that the prevalence of GNB among the patients is associated with duration of hospitalization. This agrees with the reports by LeFrock et al. (1979) and Fainstein et al.(1981), but contrasts with the report by Irwin et al. (1982).

Patients may acquire these flora from their own fecal flora (Redman and Lockey,1967; LeFrock et al.,1979) as a result of the impairment of the immunological responses (Gilman et al.,1982). Hospital environment may also be

a source for transmission among the patients. Palmer (1984) suggested that transmission among patients may be traced to the hands of the hospital personnel (especially Staphylococcus aureus and GNB), medical apparatus (especially Serratia and Pseudomonas) and food source such as Pseudomanas and Acinetobacter. Although medical personnel have low carriage rates, they may be important source for the transmission of GNB among the patients. Because, both the patient and hospital staff isolates were highly resistant. To corroborate these possible factors further investigation would be required.

Since GNB may be frequently found in the pharynx of patients without pneumonia, sputum cultures obtained from patients may not be reliable means for the diagnosis of Gram-negative bacillary pneumonia. Such pharyngeal carriage of GNB may on the other hand predispose the patient to pneumonia (Johanson et al.,1972).

In agreement with the findings of Rosenthal and Tager (1972), pharyngeal colonization with GNB among healthy subjects in this study was not sex associated. The same is true among patients.

In the present study age was not an important factor for the increase in prevalence of GNB in the pharynx. However, studies made by Tillotson and Finland (1969), Jarstrand and Tunevall (1976), and Valenti et al.(1978) have shown that elderly subjects (>60 years) have higher colonization rates. Comparison with those studies was not possible,because,the subjects studied here were generally younger,mostly below 40 years old.

The frequent isolates in this study were Klebsiella, Enterobacter, Pseudomonas and Acinetobacter species similar to the findings of Hable et al.(1971), Rosenthal and Tager (1975), Mackowiak et al.(1978), and Philpot et al.(1980).

The frequency of Klebsiella isolates (49%) from the younger population (students) was higher than the rest of the groups studied (32-34%). However, previous studies have shown that the high rate of pharyngeal carriage of Klebsiella species is associated with alcoholism (Fuxench-Lopez and Ramirez-Ronda,1978), old age (Valenti et al.,1978) and hospitalization or debility (Tillotson and Finland,1969; Pollack et al.,1972; Irwin et al.,1982). The high frequency of Klebsiella spp. among the younger population in the present study might have been also due to smoking or drinking habits. But this could not be verified.

In the present study, it was single pharyngeal specimen for each individual that was processed for culture. It was thus difficult therefore to determine whether the isolates were members of "transient" or "resident" flora. Experimentally it has been demonstrated that normal subjects swallowing high concentration of GNB do not produce persistent pharyngeal colonization (Bloomfield,1921 sited by Gilman et al.,1982) suggesting that the implantation may be inhibited by the resistant normal pharyngeal flora. Other factors, such as mechanical removal or the physico-chemical or immunologic defenses may account for the elimination of the transient colonizers from the pharynx (Johanson, et al.,1969).

Since there has not been any previous study in relation to the susceptibility of pharyngeal GNB in this country a comparison is not possible. Gentamicin, polymyxin B, Kanamycin and trimethoprim-sulphamethoxazole were the most effective antibiotics against most of the isolates except Proteus (resistant to polymyxin B) and Pseudomonas (mostly resistant to kanamycin). Unlike the surgical (Gedebou et al., 1983) and blood isolates (Gedebou et al.,1984) from Tikur Anbessa hospital (Addis Ababa,Ethiopia), the hospital isolates in this study were less resistant to tetracyctine, chloramphenicol sulphadiazine and streptomycine.

The hospital staff and patient isolates (hospital isolates) were more multiply resistant than the nonhospital isolates ($P<0.01$). Multiple antibiotic resistance among the hospital strains was similar to the isolates from Tikur Anbessa hospital patients (Addis Ababa,Ethiopia) reported by Gedebou (1983) and Gedebou et al.(1983) but was higher than the isolates in Addis Ababa from various clinical sources reported by Plorde et al.(1970). The higher rate of resistance in this study and in those of Gedebou (1983) and Gedebou et.al.(1983) could be due to increased resistance in the 10 years period. Multiple antibiotic resistance to hospital strains of Klebsiella observed in this study was consistent with the finding of Gedebou (1982); however, it was higher than the rate of Klebsiella isolates from the pharynx of patients reported by Pollack et al.(1972).in U.S.A.

The high frequency of multiple antibiotic resistance of Gram-negative pharyngeal bacilli observed in this study seems to indicate the extensive use of antibiotics in the hospital and hence the associated problem of resistance to the antibiotics.

The types of resistance patterns (antibiograms) observed with hospital isolates was more variable than the nonhospital isolates. More frequent resistance antibiograms were observed with nonhospital strains than the hospital strains in contrast to the finding of Gedebou (1983) where more frequent resistance antibiograms were detected among in-patient strains than among out-patient strains.

In almost all the more frequent resistance antibiograms, there was also resistance to ampicillin, carbenicillin and cephalothin. Above 60% of the total strains of Klebsiella were resistant to ampicillin and carbenicillin. Almost all Klebsiella found today are resistant to these two antibiotics because of a common beta-lactamase (Richmond and Sykes, 1973). It is worth mentioning that the these three antibiotics may not be used as the first drug of choice for most Gram-negative bacillary infections.

VI. RECOMMENDATIONS

Based on this study, the following recommendations are made:

1. Further studies would be required to determine the factors responsible for the prevalence of Gram-negative bacilli in the throat of normal subjects or the increase in prevalence of these flora among patients.
2. The finding further stresses that due to the possible contamination by the pharyngeal GNB, positive sputum cultures should be interpreted in the light of clinical diagnosis.
3. The high frequency of multiple antibiotic resistance of the isolates is an indication of the wide use of the respective antibiotics in and outside the hospitals. The availability of antibiotics "over the counter" and inappropriate prescription should be restricted.

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