J. Hyg., Camb. (1986), **97**, 247–253
Printed in Great Britain

Aerobic Gram-negative pharyngeal bacilli of adult Ethiopians: carrier rates and antibiograms

BY YOHANNES MENGISTU*

Department of Biology, Faculty of Science, Addis Ababa University, Addis Ababa, Ethiopia

AND MESSELE GEDEBOU

Department of Medical Microbiology and Parasitology, Faculty of Medicine, Addis Ababa University, Addis Ababa, Ethiopia

(*Received 3 February 1986; accepted 7 April 1986*)

SUMMARY

One thousand pharyngeal swab specimens were processed for aerobic culture to determine the carriage rate of Gram-negative bacilli (GNB). The isolates were identified and their sensitivity determined to 11 antibacterial drugs following standard techniques.

Similar pharyngeal carriage rates of GNB were found among the various groups of healthy subjects. Patients had higher colonization rates (27%) than healthy subjects (16%). The increase in prevalence of GNB seemed to be associated with underlying diseases and duration of hospitalization.

Klebsiella (36%) was the most frequent genus amongst the 215 isolates of GNB followed by pseudomonas (13%), enterobacter (13%) and acinetobacter (10%). Others were less frequently isolated.

Over 70% of all isolates were resistant to ampicillin (79%) and carbenicillin (72%); 55, 45 and 43% were resistant to cephalothin, tetracycline and streptomycin, respectively. The great majority of the strains were sensitive to the remaining six drugs.

The hospital isolates were more resistant than the non-hospital isolates to most drugs tested. The hospital strains were also more often multiply resistant (89%) than the non-hospital strains (60%). Sixty-five different resistance antibiograms of 1–10 drugs were observed among 191 strains. More varied types of antibiograms were observed among hospital strains.

The high frequency of multiple drug resistance of the isolates is an indication of the extensive use of antibacterial drugs, indicating the need for a policy for judicious use of drugs.

* Present address: Department of Biology, Asmara University, Asmara, Ethiopia.

INTRODUCTION

Under normal conditions, pharyngeal colonization with aerobic and facultative anaerobic Gram-negative bacilli (GNB) is infrequent (Johanson, Pierce & Sanford, 1969; Pierce & Sanford, 1974; Rosenthal & Tager, 1975). However, a high rate of colonization with Gram-negative bacilli (GNB) occurs in severely ill patients (Johanson, Pierce & Sanford, 1969; Le Frock, Ellis & Weinstein, 1979), elderly subjects (Valenti, Trudell & Bentley, 1978), those under antibiotic treatment (Le Frock, Ellis & Weinstein, 1979; Jarstrand & Tunevall, 1976), malnourished children (Gracey *et al.* 1973; Gilman *et al.* 1982), and in alcoholic and diabetic patients (Mackowiak *et al.* 1978; Fuxench-Lopez & Ramirez-Ronda, 1978; Mackowiak, Martin & Smith, 1979).

Most bacterial pneumonias are thought to be due to the flora that inhabit the pharynx. The high prevalence of pharyngeal colonization with GNB may thus predispose the patients to the development of Gram-negative bacillary pneumonia (Johanson *et al.* 1972).

In Ethiopia, there has not been any investigation of the pharyngeal flora and this study is to determine the types and frequencies and antibiograms of aerobic Gram-negative pharyngeal flora among Ethiopian patients and healthy subjects.

MATERIALS AND METHODS

Study groups

Four groups of subjects were studied in Addis Ababa, Ethiopia. Group one comprised 300 healthy students of Addis Ababa University with a mean age of 19·5 years; group two, 303 healthy adult employees of Berhanena-Selam Printing Press with a mean age of 31·5 years, and group three, 200 healthy staff of Tikur Anbessa and Yekatit 12 hospitals with a mean age of 29·5 years. Group 4, included 197 hospitalized patients, from the two hospitals, with a mean age of 37 years.

A questionnaire was administered to each participant. The apparently healthy subjects were excluded from the study if they had any type of chronic disease or respiratory infection or had used antibacterial drugs during the preceding 4 weeks. Clinical data for patients were obtained from ward charts including days of hospitalization, diagnoses and drug treatment.

Collection of specimens

Pharyngeal specimens were collected with sterile cotton-tipped swabs moistened in sterile 0·85 % saline solution. To avoid contamination, the tongue was depressed with sterile tongue depressor.

Isolation and identification of bacteria

The swab specimens were inoculated on MacConkey agar immediately or within 1 h of collection. As control, swabs immersed in the different batches of normal saline solution were similarly inoculated on the same type of medium. Plates were incubated aerobically at 37 °C overnight or if no growth, for 48 h. Isolates were identified by standard techniques (Edwards & Ewing, 1972; Lennette *et al.* 1980) using either Oxoid or Difco dehydrated products.

Drug sensitivity testing

All Gram-negative isolates were tested using 11 drug disks (bioMerieux): ampicillin (10 μg), carbenicillin (50 μg), cephalothin (30 μg), chloramphenicol (30 μg), gentamicin (10 μg), kanamycin (30 μg), polymyxin B (300 units), streptomycin (10 μg), sulphadiazine (1 mg), tetracycline (30 μg) and trimethoprim-sulphamethoxazole (25 μg). The standardized agar disk diffusion technique (Bauer *et al.* 1966) was followed and the diameters of the zone of inhibition were accordingly interpreted as intermediate, sensitive or resistant. Two standard reference strains, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were routinely tested as controls.

RESULTS

A total of 1000 pharyngeal specimens was collected from the various groups. The carrier rates of GNB among the different study groups are shown in Table 1. There was no significant difference between the carrier rates of non-hospital (16·3 %) and hospital-associated (13·5 %) healthy subjects ($P > 0{\cdot}05$). The isolation rate of GNB was significantly higher in patients (27·4 %) than in the healthy subjects (15·6 %) as a whole ($P < 0{\cdot}05$).

In all the subjects studied, the prevalence of GNB was not associated with either sex or age. Among 569 males, 101 (17·8 %) and among 431 females, 78 (18·1 %) were carriers. The mean age of the subjects from whom GNB were isolated was similar to that of the groups as a whole.

Ninety-four of the 197 patients had taken antibacterial drugs. The carrier rates were similar in both treated and untreated groups, 28·7 and 26·2 %, respectively. Gram-negative pharyngeal carriage rates among the patients seem to be positively correlated with the duration of hospitalization (Table 2).

The types and frequencies of GNB are shown in Table 3. Klebsiella was the most frequent (36·3 %), followed by pseudomonas (13·5 %), enterobacter (13·0 %), acinetobacter (10·2 %), *Escherichia coli* (9·3 %) and others of lower frequency. Some species were isolated only from some of the groups of subjects studied.

Over 90 % of the total isolates were sensitive to gentamicin and polymyxin B. Kanamycin, sulphadiazine and trimethoprim-sulphamethoxazole were effective against 91–94 % of non-hospital and 57–74 % hospital strains. Chloramphenicol, streptomycin and tetracycline were effective against 70–80 % of the non-hospital strains, while only 32, 43 and 63 % of the hospital strains were sensitive to these drugs, respectively. Ampicillin, carbenicillin and cephalothin were the least effective against the majority of isolates; only 30–55 % of the non-hospital and 11–34 % of the hospital strains were sensitive to these three drugs.

About 4 % of the non-hospital strains were fully sensitive to all 11 antibacterial drugs tested but no single hospital strain was found to be sensitive to all. About 16 % of the non-hospital strains and 1 of the 102 hospital isolates showed intermediate sensitivity to one or more of the drugs.

Resistance to one or more drugs was thus detected in 99 % and 79·6 % of the hospital and non-hospital isolates, respectively. Multiple drug resistance (resistance

Table 1. *Carriage rates of Gram-negative bacilli among the different study groups*

Study group	Specimens	Positive cultures	
Healthy subjects...	No.	No.	%
Non-hospital associated			
Students	300	45	15·0
Employees of BSP	303	53	17·5
Hospital associated staff	200	27	13·5
All healthy subjects	803	125	15·6
Hospitalized patients	197	54	27·4
Total	1000	179	17·9

Table 2. *Prevalence of GNB in relation to duration of hospitalization*

	Duration of hospitalization on day of swabbing			
	3–7	8–21	> 21	Total
No. of subjects	53	65	79	197
Positive cultures				
No.	10	19	25	54
Per cent	19	29	32	27·4

to two or more drugs) among the non-hospital strains (60·2 %) was significantly lower than that among the hospital strains (92·2 %) ($P < 0·05$).

A wide variety of antibiograms (65 types) was observed among the 191 resistant strains. The hospital strains showed more types of antibiogram (51 types) than the non-hospital strains (25 types).

Double resistance was the most frequent (28 %); the commonest was ampicillin-carbenicillin combination observed in 38 (49 %) of 78 klebsiella isolates. About 16 % of the total isolates showed triple resistance antibiograms among which the most frequent was ampicillin-cephalothin-polymyxin B resistance observed in 7 of 12 proteus isolates.

Resistance antibiograms to four, five and six drugs together comprised 24·2 % of the total isolates. Resistance to seven and more drugs was encountered almost entirely among hospital strains, mostly among *Pseudomonas* species.

DISCUSSION

The isolation rates of GNB among hospital staff and non-hospital associated healthy subjects were similar to the rates reported by Johanson, Pierce & Sanford (1969). The carrier rate among healthy subjects in this study (15·6 %) was similar to the 14 % and 18 % carrier rates reported from Puerto Rico (Fuxench-Lopez & Ramirez-Ronda, 1978) and USA (Rosenthal & Tager, 1975; Mackowiak *et al.* 1978). It was, however, higher than the 2–11 % rates of controls reported from Australia (Philpot, McDonald & Chai, 1980) and from USA (Johanson, Pierce &

Table 3. *Types and frequencies of pharyngeal Gram-negative bacilli*

Gram-negative bacterial isolates	Students (no.)	BSP employees (no.)	Hospital staff (no.)	Hospitalized patients (no.)	All subjects No. (%)
Klebsiella pneumoniae	17	12	5	19	53 (24·7)
K. ozaenae	10	7	6	2	25 (11·6)
Enterobacter aerogenes	2	5	1	2	10 (4·7)
E. hafniae	1	1	1	2	5 (2·3)
E. liquefaciens	1	—	—	5	6 (2·8)
E. cloacae	2	1	2	2	7 (3·3)
Escherichia coli	3	5	4	8	20 (9·3)
Citrobacter freundii	4	—	1	4	9 (4·2)
Proteus vulgaris	—	2	2	—	4 (1·9)
P. mirabilis	—	—	—	2	2 (0·9)
P. morganii	2	2	—	1	5 (2·3)
P. rettgeri	—	—	—	1	1 (0·5)
Serratia marcescens	—	1	2	2	5 (2·3)
Pseudomonas aeruginosa	3	5	1	5	14 (6·5)
Pseudomonas spp.	2	4	2	7	15 (7·0)
Acinetobacter spp.	6	9	2	5	22 (10·2)
Moraxella spp.	—	—	3	—	3 (1·4)
Alcaligenes faecalis	—	1	2	—	3 (1·4)
Unidentified GNB	1	4	—	1	6 (2·8)
Total GNB	54	59	34	68	215 (100)

BSP, Berhanena Selam Printing Press; GNB, Gram-negative bacilli;
—, not isolated
Percentages are out of the total number of isolates.

Sanford, 1969; Rahal *et al.* 1970; Hable, Washington & Herrmann, 1971). Rates as high as 24 % have been reported from USA (Mackowiak, Martin & Smith, 1979) and 36 % from Malaysia (Philpot, MacDonald & Chai, 1980).

Differences in the isolation rates have been attributed to various factors such as climate, food preference and other social habits (Philpot, McDonald & Chai, 1980). However, Gilman *et al.* (1982) have shown that climate is not an important factor. Different sampling and culture methods may also account for the varying prevalence rates in the different studies. Rosenthal & Tager (1975) have recommended the use of selective enrichment broth which was not used in this study.

The increase in prevalence of GNB among our hospital patients was not associated with antibiotic treatment thus agreeing with Irwin *et al.* (1982), but in contrast to the reports of Tillotson & Finland (1969) and Pollack *et al.* (1972). As is often the case, our patients might have taken antibiotics before admission to hospital which may have facilitated the colonization of the resistant bacteria as hospitalization is prolonged.

Tillotson & Finland (1969) and Valenti *et al.* (1978) have indicated that elderly subjects (> 60 years) have higher colonization rates. The lack of such variation with age in our study cannot be contrasted since our subjects were generally younger, mostly below 40 years old.

There has not been any previous report to compare the antibiograms in this study with those of the pharyngeal GNB from other carriers in this country.

However, the hospital isolates were less resistant to tetracycline, chloramphenicol, streptomycin and sulphadiazine than the clinical isolates from Tikur Anbessa Hospital (Gedebou, Tassew & Azene, 1983, 1984).

The rate of multiple antibiotic resistance amongst pharyngeal GNB was similar to the rates of non-pharyngeal clinical isolates recently reported from one of the hospitals in our study (Gedebou, 1983; Gedebou, Tassew & Azene, 1983). It is however higher than rates for clinical isolates reported by Plorde *et al.* (1970). The higher rates of resistance in the latter study could be a reflection of the increase in resistance rates in the 13 year period, resulting from the extensive use of antibiotics in the hospitals.

Multiple pharyngeal cultures done at different periods would be required to determine whether the GNB represented resident or transient flora. The findings in this study stress that the isolation of GNB from sputum culture must be considered in the light of clinical diagnoses. Also, the need to institute a policy for rational use of antibacterial drugs is clearly indicated by the high frequency of multiple drug resistant strains.

We are indebted to the staff of Tikur Anbessa and Yekatit 12 hospitals and the Bacteriology Department of the National Health Research Institute, Addis Ababa for their assistance. We thank all volunteers who participated in the study. The Swedish Agency for Research Co-operation with Developing Countries (SAREC) is gratefully acknowledged for its financial assistance.

REFERENCES

BAUER, A. W., KIRBY, W. M. M., SHERRIS, J. C. & TURK, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology* **45**, 493–496.

EDWARDS, P. R. & EWING, W. M. (1972). *Identification of Enterobacteriaceae*, 3rd ed. Minneapolis, Minnesota: Burgess Publishing Company.

FUXENCH-LOPEZ, Z. & RAMIREZ-RONDA, C. H. (1978). Pharyngeal flora in ambulatory patients: prevalence of Gram-negative bacilli. *Archives of Internal Medicine* **138**, 1815–1816.

GEDEBOU, M. (1983). Urinary tract infections: aetiologic patterns and resistance. *Ethiopian Medical Journal* **21**, 3–13.

GEDEBOU, M., TASSEW, A. & AZENE, G. (1983). Frequency and resistance patterns of bacterial isolates from surgical patients in a teaching hospital of Addis Ababa. *Tropical and Geographical Medicine* **35**, 133–138.

GEDEBOU, M., TASSEW, A. & AZENE, G. (1984). Blood culture isolates from an Addis Ababa hospital: frequency and antibiotic sensitivities. *East African Medical Journal* **61**, 190–196.

GILMAN, R. H., BROWN, K. H., GILMAN, J. B., GAFFAR, A., ALAMGIR, S. M., KIBRIYA, A. K. M. & SACK, R. B. (1982). Colonization of the oropharynx with Gram-negative bacilli in children with severe protein calorie malnutrition. *American Journal of Clinical Nutrition* **36**, 284–289.

GRACEY, M., STONE, D. E., SUHARJONO & SUNOTO (1973). Oropharyngeal microflora in malnourished children. *Australian Paediatric Journal* **9**, 260–262.

HABLE, K. A., WASHINGTON, J. A. & HERRMANN, E. C. (1971). Bacterial and viral throat flora: comparison of finding in children with acute respiratory tract disease and in healthy controls during winter. *Clinical Pediatrics* **10**, 199–202.

IRWIN, R. S., WHITAKER, S., PRATTER, M. R., MILLIARD, C. E., TARPEY, J. T. & CORWIN, R. W. (1982). The transiency of oropharyngeal colonization with Gram-negative bacilli in residents of a skilled nursing facility. *Chest* **81**, 31–35.

JARSTRAND, C. & TUNEVALL, G. (1976). Colonization and clinical superinfection with Gram-negative bacilli in influenza. *Scandinavian Journal of Infectious Diseases* **8**, 229–235.

JOHANSON, W. G., PIERCE, A. K. & SANFORD, J. P. (1969). Changing pharyngeal bacterial flora of hospitalized patients: emergence of Gram-negative bacilli. *New England Journal of Medicine* **281**, 1137–1140.

JOHANSON, W. G., PIERCE, A. K., SANFORD, J. P. & THOMAS, G. D. (1972). Nosocomial respiratory infections with Gram-negative bacilli: the significance of colonization of the respiratory tract. *Annals of Internal Medicine* **77**, 701–706.

LE FROCK, J. L., ELLIS, C. A. & WEINSTEIN, L. (1979). The relationship between aerobic fecal and oropharyngeal microflora in hospitalized patients. *American Journal of Medical Sciences* **277**, 275–280.

LENNETTE, E. H., BALOWS, A., HAUSLER, W. J. & TRUANT, J. P. (1980). *Manual of Clinical Microbiology*, 3rd ed. Washington, D.C.: American Society for Microbiology.

MACKOWIAK, P. A., MARTIN, R. M., JONES, S. R. & SMITH, J. W. (1978). Pharyngeal colonization by Gram-negative bacilli in aspiration-prone persons. *Archives of Internal Medicine* **138**, 1224–1227.

MACKOWIAK, P. A., MARTIN, R. M. & SMITH, J. W. (1979). Role of bacterial interference in the increased prevalence of oropharyngeal Gram-negative bacilli among alcoholics and diabetics. *American Review of Respiratory Diseases* **120**, 589–593.

PHILPOT, C. R., MCDONALD, P. J. & CHAI, K. H. (1980). Significance of enteric Gram-negative bacilli in the throat. *Journal of Hygiene* **85**, 205–210.

PIERCE, A. K. & SANFORD, J. P. (1974). Aerobic Gram-negative bacillary pneumonias. *American Review of Respiratory Diseases* **110**, 647–658.

PLORDE, J. J., ENG, S. C., WRIGHT, L. J. & DEBESAI, A. (1970). Antibiotic sensitivities of common bacterial pathogens isolated in Addis Ababa. *Ethiopian Medical Journal* **8**, 107–118.

POLLACK, M., NEIMAN, R. E., REINHARDT, J. A., CHARACHE, P., JETT, M. P. & HARDY, P. H. (1972). Factors influencing colonization and antibiotic resistance patterns of Gram-negative bacteria in hospital patients. *Lancet* i, 668–671.

RAHAL, J. J., MEADE, R. H., BUMP, C. M. & REINAUER, A. J. (1970). Upper respiratory tract carriage of Gram-negative enteric bacilli by hospital personnel. *Journal of American Medical Association* **214**, 754–756.

ROSENTHAL, S. & TAGER, I. B. (1975). Prevalence of Gram-negative rods in the normal pharyngeal flora. *Annals of Internal Medicine* **83**, 355–357.

TILLOTSON, J. R. & FINLAND, M. (1969). Colonization and clinical superinfection of the respiratory tract complicating antibiotic treatment of pneumonia. *Journal of Infectious Diseases* **119**, 597–624.

VALENTI, W. M., TRUDELL, R. G. & BENTLEY, D. W. (1978). Factors predisposing to oropharyngeal colonization with Gram-negative bacilli in the aged. *New England Journal of Medicine* **298**, 1108–1111.