J. Hyg., Camb. (1980), 85, 205
Printed in Great Britain

Significance of enteric Gram-negative bacilli in the throat

By C. ROSS PHILPOT, P. J. McDONALD*
Department of Clinical Microbiology, School of Medicine, Flinders University, Bedford Park, South Australia, 5042
AND K. H. CHAI
Department of Medical Microbiology, University of Malaya, Kuala Lumpur, Malaysia

(*Received* 20 *November* 1979)

SUMMARY

Pharyngeal micro-organisms of 131 Australian and Malaysian children and adults were compared by analysis of aerobic culture of throat swab specimens. Enteric Gram-negative bacilli were commonly isolated in small numbers from Malaysian adults whether they had sore throats (28%) or not (36%), but were detected in only 9% of Australian adults without sore throats and in only 12% and 4% of Malaysian children with and without sore throats respectively. In other respects microbiological findings were similar in the different groups of subjects studied.

It is concluded that the pharyngeal carriage rate of enteric Gram-negative bacilli may differ substantially between different groups of normal individuals. Our findings also suggest that these micro-organisms do not have a pathogenic role in pharyngitis.

INTRODUCTION

International comparative studies of normal microflora of the pharynx are few. Studies of the bacteria found in sore throats mainly concern the accepted pathogens *Streptococcus pyogenes* and *Corynebacterium diphtheriae* in temperate climates. However, in South-East Asian countries, routine laboratory experiences with throat swab specimens suggest a higher isolation rate of Enterobacteriaciae and species of *Pseudomonas* than in temperate climates. Furthermore, personal observations of clinical practices in these countries indicate that the detection of these enterobacteria (Wilson & Miles, 1975) in throat swab specimens from subjects with sore throats leads to the use of expensive and potentially harmful antibacterial drugs. Previous reports have suggested that a high isolation rate of enterobacteria from the adult upper respiratory tract is a consequence of previous infection (Weinstein, Goldfield & Chang, 1954; Jarstrand & Tunevall, 1976), alcoholism (Fuxench-Lopez & Ramirez-Ronda, 1978), or illness requiring hospi-

* With assistance from Wendy A. Graham, Julie Forsythe, Peter Ingham, Gary Shanks, Dorothy Jones, Peter Papay and Robert Van den Berg, School of Medicine, Flinders University of South Australia.

0022-1724/80/0104-1979 $01.00

talization (Johanson, Pierce & Sanford, 1964; Pollack *et al.* 1972), and not a finding in 'normal' populations.

The aims of the present investigation were to compare the pharyngeal flora of non-institutionalized residents of temperate and tropical climates, and to elucidate the possible role of enterobacteria in pharyngitis. This work was undertaken as an extension of an elective project on normal flora of the human body by third year medical students.

MATERIALS AND METHODS

A total of 131 subjects in five groups were studied. Group (i) comprised 31 healthy adult employees of a plastic and metals factory in Adelaide, South Australia; group (ii) 25 healthy adult Malaysian school staff; group (iii) 25 healthy Malaysian school children; group (iv) 25 Malaysian adults seeking medical attention because of sore throats; group (v) 25 Malaysian school-age children complaining of sore throat and attending an outpatient department. Persons in group (i) were studied during September 1976 and persons in groups (ii) to (v) were studied in Kuala Lumpur, Malaysia, during May 1977. All those with sore throats had reddened fauces. Similar materials, methods and investigating personnel were used in both Adelaide and Kuala Lumpur. Horse blood, human serum, identification disks and biochemical fermentation test reagents were taken to Malaysia in one refrigerated container and the culture media were prepared by the staff of the University of Malaya.

A sterile cotton-wool-tipped swab was moistened in sterile 0·9 % saline and stroked across the pharynx and both tonsillar fossae without touching the tongue. It was then inoculated onto culture plates within 1 h in a manner which allowed quantitation (Rotheram, 1975). Media used were horse blood agar, chocolate agar and MacConkey's medium in all cases, and also Sabouraud's standard solid medium, Thayer-Martin medium and tellurite medium in Malaysia. Bacteria were identified by standard laboratory methods (Cowan, 1974; Lennette, Spaulding & Truant, 1974), including the API 20 system (Analytab Products Inc., New York, 20 biochemical tests) for enteric Gram-negative bacilli.

The χ^2 test with Yates' continuity correction (Armitage, 1971) was used to determine the significance of observed differences between isolation rates in the different groups of subjects.

RESULTS

Isolation rates of the various throat micro-organisms are shown in Table 1. The isolation rate of enterobacteria was significantly different between Malaysian adults (36 %) and Malaysian children (4 %) ($P < 0{\cdot}05$) and between Malaysian adults and Australian adults (9 %) ($P < 0{\cdot}05$). There was no significant difference in isolation rate of enterobacteria between Malaysian adults with pharyngeal symptoms (28 %) and those without (36 %), and in each case the numbers of these bacteria detected were not great (see below).

Streptococci of the 'viridans' group were found most frequently and were the only micro-organisms detected in 50 % or more of the subjects in all of the five

Table 1

	No pharyngeal symptoms			Sore throats	
	Group (i) Adults ($n=31$)	Group (ii) Adults ($n=25$)	Group (iii) Children ($n=25$)	Group (iv) Adults ($n=25$)	Group (v) Children ($n=25$)
Klebsiella species	3*	12	0	12	8
Serratia species	0	16	0	8	0
Enterobacter species	0	4	0	8	0
Providencia species	0	8	4	0	4
Edwardsiella species	0	4	0	4	0
Escherichia coli	0	4	0	0	0
Proteus species	6	0	0	0	0
Pseudomonas species	0	0	0	0	0
All enteric Gram-negative bacilli	9	36	4	28	12
'Viridans' streptococci	61	68	92	52	80
Staphylococcus epidermidis	35	48	76	32	60
Staph. aureus	29	4	4	8	12
Streptococcus pneumoniae	0	0	0	0	0
Strept. pyogenes	16	0	0	4	0
Beta-haemolytic streptococci not pyogenes	35	16	8	16	8
'Faecal' streptococci	10	0	0	0	0
Non-haemolytic streptococci	23	28	12	20	24
Micrococcus species	0	8	36	20	24
All Gram-positive cocci	100	92	92	76	88
Corynebacterium diphtheriae	0	0	0	12	0
Corynebacterium species	10	32	44	44	48
Neisseria meningitidis	0	0	0	0	0
Neisseria species	23	88	76	92	84
Haemophilus influenzae	19	4	0	4	0
Haemophilus species	0	44	20	36	24
Candida albicans	0	0	4	0	0

* Numbers refer to percentage of positive cultures in each group.
Group (i): adults in South Australia without pharyngeal symptoms.
Group (ii): adults in Malaysia without pharyngeal symptoms.
Group (iii): children in Malaysia without pharyngeal symptoms.
Group (iv): adults in Malaysia with sore throats.
Group (v): children in Malaysia with sore throats.

groups. Other micro-organisms consistently found in all five groups were *Staphylococcus epidermidis* (32–76%), *Neisseria* species other than meningococci (23–92%), *Corynebacterium* species not *C. diphtheriae* (10–48%), beta-haemolytic streptococci not group A (8–35%), *Staphylococcus aureus* (4–29%) and non-haemolytic streptococci (12–28%). Micro-organisms occasionally present in the five groups included micrococci (0–36%), *Haemophilus influenzae* (0–19%), other *Haemophilus* species (0–44%), and *Corynebacterium diphtheriae* (0–12%). Meningococci and pseudomonads were not detected, and optochin disk susceptibility testing of selected alpha-haemolytic colonies failed to demonstrate pneumococci. *Candida albicans* (0–4%) was the only fungal isolate. *Streptococcus pyogenes* was

isolated in 16% of symptom-free adults in Adelaide and in 0–4% of the Malaysian groups of subjects.

Quantitative assessment of colonies on primary isolation revealed that the single isolation of *Streptococcus pyogenes* was equivalent to 10^6 colony forming units per ml of pharyngeal secretion (c.f.u./ml) and the growth of *Corynebacterium diphtheriae* equivalent to 10^4–10^5 c.f.u./ml. Viridans streptococci, *Staphylococcus epidermidis* and *Neisseria* species were detected in numbers of 10^4–10^7 c.f.u./ml, and growths of *Staphylococcus aureus*, non-haemolytic streptococci, *Micrococcus* species and *Haemophilus* species not *H. influenzae* mainly 10^4–10^5 c.f.u./ml. Growths of enterobacteria, *Corynebacterium* species not *C. diphtheriae*, and non-group A beta-haemolytic streptococci were detected in numbers of 10^3–10^5 c.f.u./ml.

DISCUSSION

The predominant bacteria in all groups of subjects in both countries were Gram-positive cocci, particularly the 'viridans' group of streptococci and *Staphylococcus epidermidis* (Table 1). The isolation rates of these and other bacteria were generally similar to those recorded in standard reference works on the subject (Wilson & Miles, 1975; Sonnenwirth, Gibbon & Socransky, 1973; Isenberg & Painter, 1974) with the one exception that enterobacteria were found more commonly amongst Malaysian adults in the present study.

About one-third of the Malaysian adults were found to harbour enterobacteria whether they had sore throats or not. Rosenthal & Tager (1975) investigated 100 subjects without respiratory disease and found only a 6% prevalence of enterobacteria in the temperate climate of Massachusetts, U.S.A. Hable, Washington & Herrmann (1971) reported a similar isolation rate in 490 healthy children in the temperate climate of Minnesota, U.S.A. The commonest enterobacteria found by these two groups of workers were species of *Escherichia*, *Klebsiella*, *Enterobacter*, *Pseudomonas*, *Acinetobacter*, *Serratia* and *Proteus*. They reported a wide range of these bacteria, as was found in the present study (Table 1).

Isolation rates of greater than 15% have generally been found in debilitated subjects (Weinstein *et al.* 1954; Jarstrand & Tunevall, 1976; Fuxench-Lopez & Ramirez-Ronda, 1978; Johanson *et al.* 1964; Pollack *et al.* 1972). Our subjects had not been in hospital recently and were not alcoholics, nor had they been taking long-term antimicrobial therapy. Furthermore, the significant difference in isolation rates between Malaysian adults and children indicates that climatic factors (such as temperature and humidity) cannot be the only reasons for our findings. It is tempting to ascribe the routine laboratory isolation of enteric bacteria to 'overgrowth' during transport to the laboratory. However, in our investigations care was taken to avoid proliferation of enteric Gram-negative bacteria by inoculating the swabs onto culture media immediately after specimens were taken.

Food preferences or other social habits are probably as important as the factors considered above. Many decades of study have given rise to considerable debate over whether dietary factors can influence the normal flora of the gastro-

intestinal tract of different community populations (Gorbach *et al.* 1967) but recent investigations by Bettelheim *et al.* (1977) have clearly shown that diet can influence the carriage of different serotypes of *Escherichia coli* in carefully studied individuals. There is evidence that plant foods such as raw fruit, vegetables and salads (Cooke *et al.* 1970) and the meat of cattle, pigs and chickens (Shooter *et al.* 1970) can be dietary sources of *Escherichia coli*. Gracey, Ostergaard & Beaman (1979) suggested 'faecal–oral spread' as an explanation for their finding of a high isolation rate of what they referred to as 'faecal micro-organisms' in some groups of children. Further studies will be required to determine which factors are important in giving rise to a high prevalence of various Gram-negative bacilli in the throats of specific groups of normal as well as unwell subjects.

The low isolation rate of beta-haemolytic streptococci of Lancefield Group A (*Streptococcus pyogenes*) from subjects with sore throats may at first sight seem unexpected. However, it has previously been found that the prevalence of pharyngeal carriage of *Streptococcus pyogenes* in Kuala Lumpur is lowest (0–5 %) at the time of the year our study was undertaken (Chen, Dugdale & Puthucheary, 1972). Furthermore, retrospective analysis of the culture results of throat swabs processed in the Department of Medical Microbiology, University of Malaya, in the months before, during and after the present study revealed that of 56 specimens from patients with upper respiratory tract disease (27 children, 29 adults), there were only 2 isolations of beta-haemolytic streptococci, both *Streptococcus pyogenes*. This isolation rate of 4 % is very similar to that in our field study. The heavy growth of *Streptococcus pyogenes* isolated from an adult with pharyngitis validates the detection system used for this micro-organism.

These observations challenge common assumptions that a high rate of pharyngeal carriage of enteric Gram-negative bacilli is exclusively associated with hospitalization or debility.

Acknowledgements are gratefully made to Abbott Australasia Pty. Ltd, for financial support and organizational help; to Dr A. S. Cameron for help in the Adelaide field study; to Wendy H. Graham for secretarial assistance; to Dr John Finlay-Jones for statistical analyses and to Dr S. D. Puthucheary for throat swab surveillance data from the Department of Medical Microbiology, University of Malaya.

REFERENCES

Armitage, P. (1971). *Statistical methods in Medical Research*. Oxford: Blackwell Scientific Publications.

Bettelheim, K. A., Cooke, E. M., O'Farrell, S. & Shooter, R. A. (1977). The effect of diet on intestinal *Escherichia coli*. *Journal of Hygiene* **79**, 43–5.

Chen, S. T., Dugdale, A. E. & Puthucheary, S. D. (1972). Beta-haemolytic streptococcal carriers among normal school children. *Tropical and Geographical Medicine* **24**, 257–64.

Cooke, E. M., Shooter, R. A., Kumar, P. J., Rousseau, S. A. & Foulkes, A. L. (1970). Hospital food as a possible source of *Escherichia coli* in patients. *Lancet* i, 436–7.

Cowan, S. T. (1974). *Cowan and Steele's Manual for the Identification of Medical Bacteria*. Cambridge University Press.

FUXENCH-LOPEZ, Z. & RAMIREZ-RONDA, C. H. (1978). Pharyngeal flora in ambulatory alcoholic patients. Presence of Gram-negative bacilli. *Archives of Internal Medicine* **138**, 1815–16.

GORBACH, S. L., NAHAS, L., LERNER, P. I. & WEINSTEIN, L. (1967). Studies of intestinal microflora. I. Effects of diet, age, and periodic sampling on numbers of fecal microorganisms in man. *Gastroenterology* **53**, 845–55.

GRACEY, M., OSTERGAARD, P. & BEAMAN, J. (1979). Oropharyngeal microflora in aboriginal and non-aboriginal Australian children. An indicator of environmental contamination. *Medical Journal of Australia* **2**, 212–14.

HABLE, K. A., WASHINGTON II, J. A. & HERRMANN, E. C. (1971). Bacterial and viral throat flora. Comparison of findings in children with acute upper respiratory tract disease and in healthy controls during winter. *Clinical Pediatrics* **10**, 199–203.

ISENBERG, H. D. & PAINTER, B. G. (1974). Indigenous and pathogenic microorganisms of man. In *Manual of Clinical Microbiology*, 2nd ed. (ed. E. H. Lennette, E. H. Spaulding & J. P. Truant), pp. 45–8. Washington: American Society for Microbiology.

JARSTRAND, C. & TUNEVALL, G. (1976). Colonisation and clinical superinfection with Gram-negative bacilli in influenza. *Scandinavian Journal of Infectious Diseases* **8**, 229–35.

JOHANSON, W. G., PIERCE, A. K. & SANFORD, J. P. (1964). Changing pharyngeal bacterial flora of hospitalised patients. *New England Journal of Medicine* **281**, 1137–40.

LENNETTE, E. H., SPAULDING, E. H. & TRUANT, J. P. (1974). *Manual of Clinical Microbiology*, 2nd ed. Washington: American Society for Microbiology.

POLLACK, M., CHARACHE, P., NEIMAN, R. E., JETT, M. P., REINHARDT, J. A. & HARDY, P. J. JR. (1972). Factors influencing colonisation and antibiotic-resistance patterns of Gram-negative bacteria in hospital patients. *Lancet* ii, 668–71.

ROSENTHAL, S. & TAGER, I. B. (1975). Prevalence of Gram-negative rods in the normal pharyngeal flora. *Annals of Internal Medicine* **83**, 355–7.

ROTHERAM, E. B. (1975). Septic abortion and related infections of pregnancy. In *Anaerobic Bacteria: Role in Disease* (ed. A. Balows, R. M. Dehaan, V. R. Dowell & L. B. Guze), pp. 369–78. Illinois: Thomas.

SHOOTER, R. A., COOKE, F. M., ROUSSEAU, S. A. & BREADEN, A. L. (1970). Animal sources of common serotypes of *Escherichia coli* in the food of hopsital patients. *Lancet* i, 226–8.

SONNENWIRTH, A. C., GIBBON, R. & SOCRANSKY, S. (1973). Bacteria indigenous to man. *Microbiology* (ed. B. D. Davis, R. Dulbecco, H. N. Eisen, H. S. Ginsberg, W. B. Wood & M. McCarty), pp. 953–63. Maryland: Harper & Row.

WEINSTEIN, L., GOLDFIELD, M. & CHANG, T. W. (1954). Infections occurring during chemotherapy. *New England Journal of Medicine* **251**, 247–55.

WILSON, G. S. & MILES, A. A. (1975). *Topley & Wilson's Principles of Bacteriology, Virology and Immunity*. London: Arnold.