Normal Microbial Flora of the Pharyngeal Mucosa

J. Perera[1]

The Ceylon Journal of Medical Science 1993; 36: 33-37

Summary

Aerobic and facultative anaerobic microbial flora of the throat mucosa were studied in 349 healthy persons aged 5-50 years. The results identified the presence of 3 broad groups of microorganisms in the throat.

The indigenous (permanent) group consisted of *viridans* group of *streptococci* and *neisseria*, characterized by permanent (90 - 100%) colonization. They showed a broad species spectrum, associations of 2-3 species and were not affected by age.

The representatives of the facultative group, *Corynebacterium, Staphylococcus* and *Micrococcus* species were less frequent (11 - 28%). The intensity of their colonization was lower than that of the indigenous group and species spectrum narrow. Coagulase negative *Staphylococci* was significantly higher in children aged between 5 - 10 years compared to other age groups.

The group that consisted of potential pathogens or transitory group were characterized by low frequency (3 - 6%) and the intensity of colonization was lower compared to both indigenous and facultative group, the only exception being *Klebsiella/Enterobacter* group which showed a higher frequency and intensity of colonization. The nature of colonization was monospecific. The group consisted of *Streptococcus pyogenes, Streptococcus pneumoniae, Staphylococcus aureus, Haemophilus influenzae, Klebsiella* species, *Enterobacter* species. The facultative and potentially pathogenic groups were subject to age variation.

Key words: Pharynx, Microbial flora, Pathogens/Commensals.

Introduction

The common regional flora of the upper respiratory tract include, *viridans* group of *streptococci, diphtheroids,* non-pathogenic *Neisseria* species, *Staphylococcus epidermidis, Haemophilus* species, fusiform bacilli and spirochaetes.

The majority of infections in the throat are caused by viruses which account for over 70% of infections. Important bacterial pathogens in the throat include *Streptococcus pyogenes* and *Corynebacterium diphtheriae* which account for the majority of the remaining 30% acute pharyngitis cases. The potential bacterial pathogens that are commonly carried in the throat are *Haemophilus influenzae, Neisseria meningitidis* and *Staphylococcus aureus* which produce their main ill effects elsewhere in the body.

The study was conducted in order to determine the prevalence of

(a) different bacteria in the pharynx of normal healthy people.

(b) carriage of potential pathogens in the throat in people without symptoms.

Materials and Methods

The number of healthy subjects from each age group used as the study population is shown in Table 1.

Subjects who had been treated with antimicrobial agents during the previous two weeks were excluded from the study.

The throat swabs were collected by the same person from each subject and plated first on

1 Department of Microbiology, Faculty of Medicine, Colombo 8.

sheep blood agar, followed by chocolate agar and blood tellurite agar. Plates were incubated at 37°C in 5% CO_2 (candle jar) within 1 hour of collection. To facilitate the counting of colonies the blood agar plate was inoculated by rolling the swab across the same diameter of the plate twice. Streaking was carried out perpendicular to the inoculation line using a bacteriological loop. Dilution techniques were not employed.

The plates were examined after overnight incubation and different colony appearances were noted. A count of each type of colony was carried out using the blood agar plate. It was assumed that each different colony type represented a different species.

Different colony types were gram stained, and identified using the standard biochemical tests (1). Gram negative diplococci were identified into different species on results of sugar fermentation tests and on the ability to grow on nutrient agar. Alpha haemolytic *streptococci* were first tested for optochin sensitivity for identification of *S. pneumoniae*. Those that were optochin resistant and the non-haemolytic *streptococci* were further tested using biochemical tests. Those that could not be designated *viridans* group of *streptococci* were considered *streptococcus* species and not identified further. *Viridans* group of *streptococci* were further tested for sugar fermentation, arginine hydrolysis and urease production in order to speciate further (2).

Staphylococci were differentiated into *staphylococcus aureus* and coagulase negative *staphylococci* using the coagulate test (1).

Results

A total of 349 specimens were examined from different age groups. The number of specimens from which different species were isolated are shown in Table 2. The relative incidence of the species in different age groups is shown in Table 3. *Viridans* group of *Streptococci* and *Neisseria* species were the commonest bacteria found in all age groups, ie. 94% and 100% respectively. *N.flavescens* and *N.sica* were the commonest *Neisseria* species isolated from the throat. Other species were *N.subflava, N.lactamica* and *N.mucosa*. In a single specimen more than one species of *Neisseria* and *viridans* group of *streptococci* were detected. The number of colony forming units was >50/swab in 72.4% and 80% of the isolates belonging to *Neisseria* species and *viridans* group of streptococci respectively.

Staphylococcus species other than *Staphylococcus aureus* were isolated in a significantly higher number of children aged between 5 - 10 years when compared to other age groups ($P<0.001$). Intensity of colonization of *Staphylococcus* species, non group A *streptococci, Micrococcus* species and diphtheroids was lower compared to *Neisseria* species and *Streptococcus viridans*. This group could be considered the facultative group. The number of colony forming units ranged from 20 to 50/swab in 60.8% of isolates in the facultative group.

The potential pathogens isolated were *S.aureus, S.pneumoniae, H.influenzae, S.pyogenes, Klebsiella species, Enterobacter* species and *Pseudomonas aeruginosa*. *C.diphtheriae* was notably absent. *C.haemolyticum* was isolated from a 22 year old

Table 1. Age and sex distribution of the study population

Study population	Age Group Years	Sex M	Sex F	Total Number of Subjects
School children	5 – 10	51	50	101
School children	10 – 20	50	50	100
Medical students	20 – 30	57	34	91
Staff – Faculty of Medicine	30 – 40	12	09	21
Staff – Faculty of Medicine	40 – 50	12	12	24

Table 2. Number of specimens that yielded different bacterial species

Bacterial species	Number of positive Specimens	(%)
Strept viridans group	331	(94)
Neisseria species	349	(100)
Coagulase negative *staphylococci*	40	(11.4)
Diphtheroids	43	(12.3)
Micrococcus species	70	(20.0)
Non group A *Streptococci*	21	(6.0)
Staphylococcus aureus	72	(20.6)
Streptococcus pneumoniae	45	(12.8)
Haemophilus influenzae	10	(2.8)
Streptococcus pyoqenes	18	(5.1)
Haemophilus species	4	(1.1)
Klebsiella/Enterobacter sp.	97	(27.7)
Pseudomonas aeruqinosa	1	(0.3)
Corynebacterium haemolyticum	1	(0.3)

Total number of specimens studied = 349

Table 3. Incidence of different bacterial species in relation to age group (%)

Pathogen	5 - 10 yr n = 101	11 - 20 yr n = 100	21 - 30 yr n = 103	31 - 40 yr n = 21	41 - 50 yr n = 24
S. viridans	91 (90)	97 (97)	101 (96)	19 (90)	23 (96)
N. flavescens	58 (57)	54 (54)	52 (50)	13 (62)	17 (74)
N. sica	68 (67)	61 (61)	54 (52)	4 (19)	10 (42)
N. subflava	33 (33)	28 (28)	42 (41)	9 (43)	9 (39)
N. lactamica	11 (11)	18 (18)	12 (12)	1 (5)	1 (4)
N. mucosa	16 (16)	18 (18)	28 (27)	9 (43)	5 (21)
Micrococcus spp	4 (4)	28 (28)	19 (18)	8 (28)	11 (46)
Diphtheroids	21 (21)	5 (5)	12 (12)	1 (5)	4 (17)
Coagulase negative *Staphylococci*	24 (24)	7 (7)	7 (7)	1 (5)	1 (4)
Non group A, ß haemolytic					
Streptococcus sp.	9 (9)	7 (7)	4 (4)	0 (0)	1 (4)
S. aureus	28 (28)	21 (21)	16 (15)	2 (9)	5 (21)
S. pneumoniae	13 (13)	11 (11)	17 (16)	0 –	4 (17)
H. influenzae	–	2 (2)	–	6 (28)	2 (8)
Haemophilus spp	2 (2)	–	–	0	2 (8)
S. pyogenes	10 (10)	5 (5)	1 (0.9)	3 (2)	2 (8)
Kleb/Enterobacter	30 (30)	24 (24)	19 (28)	4 (19)	10 (42)
Psd. aeruginosa	–	–	–	–	–
C. haemolyticum	–	–	1	–	–

n = Total number tested in each age group

male. *H.influenzae* was isolated from 28% of the study population in the age group between 31-40 years. This is significantly higher ($P<0.01$) when compared to isolation rates of other age groups. Klebsiella species was isolated from 50% of the population in the age group 41-50 years ($P<0.05$). *S.pyogenes* was isolated from 10% and 5% of the children in the 5-10 year and 10-20 year age groups respectively. Non group A, B.haemolytic *Streptococci* were also commoner in 5-10 years age group.

The degree of colonization of potential pathogens varied among the different species. The majority of *S.aureus* (97%) and *H.influenzae* (100%) isolates showed 1-20 colony forming units (CFU)/swab (Table 4). In contrast *Klebsiella* species showed >10 CFU/swab in the majority (97.4%) of cases. *S.pyogenes* was isolated in 5.1% of the total study population and most (65%) had 1-10 CFU/swab.

Corynebacterium species (12%) were isolated (Table 2). They were less frequent, intensity of their colonization was lower and species spectrum narrow. The isolation rate of facultative group of bacteria were lower compared to a study carried out by Kolotilova *et al.* where the isolation rates ranged from 20% to 50% (3).

The potentially pathogenic group of bacteria (*S.pneumoniae, H.influenzae, S.aureus* and *Klebsiella/Enterobacter* species) were generally limited to one colonization type. In this group colonization species varied in different age groups. Notably *H.influenzae* was isolated in 28% of the 31-40 year age group. *S.pyogenes* carriage was significantly commoner (5-10%) in 5-20 year age group compared to other groups. 21% of children in the age group 1-20 years were colonized with potential respiratory pathogens ie. *H.influenzae, S.pyogenes* and *S.pneumoniae.* Colonization with *Klebsiella* species was higher

Table 4. Degree of colonization of potentially pathogenic species

Bacterial species	No. of specimens	Colony forming units/Swab 1 - 10	11 - 20	> 21
S.aureus	67	37	24	6
S.pyogenes	17	11	4	2
S. pneumoniae	12	1	9	2
Klebsiella/Enterobacter sp	78	2	39	37
H. influenzae	9	5	4	0

Discussion and Conclusions

The analysis of the study shows that normal throat flora in the asymptomatic group include 3 groups of organisms. *S.viridans* and *Neisseria* species could be considered the indigenous group. This group was characterized by permanent (>90%) and intensive colonization. They showed a broad species spectrum and associations of more than 2-3 species at a single colonization. Sex and age had no significant influence on the indigenous group.

In the facultative group, bacteria belonging to *Staphylococcus* species (11%), non group A *Streptococci* (6%), *Micrococcus* species (20%) and in the older age groups (40 - 50 years). These rates are similar to results obtained in a household study carried out in Scandinavia(4). A study carried out in Bangladesh showed a higher carriage rate (20%) of *S.pyogenes* (5).

The isolation of these potentially pathogenic organisms therefore in throat swabs from patients have to be interpreted carefully. A semiquantitative analysis of samples may provide a better guide in determining actual infection in contrast to bacterial colonization.

Acknowledgements

I am grateful to Dr. Nelun Perera for collecting specimens, Ms. Chitra Ranjithan for technical

assistance; Principals of Gothami Balika Vidyalaya and Carey College for permiting me to collect samples from school children; Ms. Shirani Hendalage for typing the manuscript and University of Colombo for financial support.

Reference

(1) Cowan ST, Steel KJ. Manual for the Identification of Medical Bacteria. Editors: GI Barrow and RK Fethem; 3rd Edition; Cambridge University Press, 1991.

(2) Barlows HD, Hausler WJ, Herrmann KL, Isenberg HD, Shadomy HJ. Manual of Clinical Microbiology, 5th edition, Washington DC; American Society for Microbiology, 1991, pp 238-257.

(3) Kolotilova LV, Akishina TM, Zargarian OP, Lomnitskaia VB, Pruzhniak OV, Lutsik TS. Normal Microflora of the pharyngeal mucosa. Antibiotiki I; Khimioterapiia (Moskva) 1989; 34 (10): 751-755.

(4) Schwan A, Eriksson M, Eriksson BM, Carlson U, Petterson E, Sandblom M, Tibblin G. Colonization with potentially pathogenic respiratory tract bacteria. A household study. Scandinavian Journal of Primary Health Care 1989; 7(4) : 203-209.

(5) Hussain T, Rahman KM. Normal aerobic bacterial flora of throat in the population of Dhaka city. Bangladesh Medical Research Council Bulletin. (Dacca) 1987; 13(1) : 8-14.