Research Paper

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Throat Isolates of β-haemolytic Streptococci, from Rural Bengal Showing Reduced Susceptibility to Many Antibiotics

Microbiology

KEYWORDS : Group A Streptococci (GAS), throat swab, Pharyngitis

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ABSTRACT

Since most of the sore throats are caused by viral infection rather than bacteria, a correct diagnosis is important to avoid unnecessary use of antibiotics. To determine the incidence, socioeconomic & environmental parameters, seasonal variation and antimicrobial susceptibility of Streptococcal sore throat, samples from 520 cases were collected from rural Bengal between September 2012 to August 2013 and processed. Group A Streptococci (GAS) were identified and further confirmation was done by Group specific antisera. Demographic and socio-environmental data were collected and antimicrobial susceptibility testing was performed. Out of 520 samples, 58 (11.15%) were GAS and 10 (1.92%) were Group CG Streptococci. The incidence was higher among 5-15 yrs., during winter season and among people living in overcrowded conditions, using chullah for cooking and in homes that included a tobacco-smoker. GAS isolates were 100% sensitive to penicillin-G while 56.8%, 67.2%, 82.7% and 79.3% sensitive to ciprofloxacin, levofloxacin, erythromycin and doxycycline respectively.

Introduction

The primary purpose of a throat culture is to isolate and identify organisms from the throat that cause infection of the posterior pharynx and tonsillar areas. More than 225 pathogens, including about 200 viruses, are responsible for upper respiratory tract infections as per the research done by Mathur et al.[1]. Since most sore throats are caused by viral infections rather than by bacteria, a correct diagnosis is important to prevent unnecessary use of antibiotics. The bacterium that most often causes a sore throat is *Streptococcus pyrogenes* or Group A beta-hemolytic streptococcus (GAS). Post-streptococcal sequelae, especially acute rheumatic fever/rheumatic heart disease , acute and chronic renal failure continue to occur in significant proportions in many parts of the world due to repeated episodes of streptococcal infections. Throat cultures are also performed to identify people who are the carriers of the organisms that may cause meningitis (*Neisseria meningitidis, Streptococcus pneumoniae*) and whooping cough (*Bordetella pertussis*) and also screening of methicillin resistance *staphylococcus aureus* (MRSA). However, prospective studies regarding incidence of GAS sore throat, its seasonal variation as well as its distribution in different age, sex, and socioeconomic groups are scanty. The present study was therefore conducted to isolate the pathogen and its sensitivity to different antibiotics in the patients of sore throat and its relation with different age, sex, socioeconomic groups, environmental factors and seasonal variation.

Material and methods

Studyarea

The study was based on a house-to-house survey as well as patients attending outdoor department (OPD) in a rural community of Budge-Budge-II block and LB Dutta hospital of the block in the district of South 24 parganas, West Bengal, India. There are 65 villages in the block with a total population of 1,90,000. The majority were engaged as daily wage labourers in the government or private sectors and lived in unhygienic and overcrowded conditions.

Sampling and sample size

The multistage random sampling technique was adopted in the study. Out of the 520 study subjects, 25% i.e. 130 patients were selected from the Out Patients Department of LB Dutta hospital. Rest i.e. 390 were selected at community level through cluster sampling method. Visits were divided into four phases and in each visit 14 study subjects from each cluster was selected. Starting in an unbiased manner, the subsequent house-holds were approached until the desired number of subjects was included/selected from each cluster.

Inclusion criteria

People of all age groups and sex suffering from Upper Respiratory tract Infection (URTI) presented with sore throat, dry cough with fever for less than 7days.

Exclusion criteria

Cough with expectoration due to lower respiratory tract infection (LRTI)/TB/asthma or COPD /CCF etc.and the patients receiving antibiotics were excluded from the study.

Data collection

A house hold list was prepared by the help of the ASHA (Accredited social health activist) volunteers. Demographic, socioeconomic as well as environmental data, obtained by interviewing the head of each household, was recorded on that structured proforma. Data were collected for a period of one year from September 2012 to August 2013, thus covering all seasons, i.e. autumn (September to November), winter (December to February) and summer months (March to May) and rainy season (June to August).

Collection and transport of samples

For collection of throat swabs the patient was asked to tilt the head back and open the mouth wide. A tongue depressor was used to hold down the tongue and the tip of the sterile swab was rubbed against posterior pharynx and tonsillar areas on both sides of the throat. The swab was then removed gently without touching the teeth, gums, or tongue. It was then placed in Amie's transport medium and transported to the laboratory within 2 to 3 hours via vaccine box at room temperature.

Laboratory investigation

The throat swabs were then inoculated on blood agar media containing 5% defibrinated sheep blood and incubated for 24 h at 37 oC. Identification of *Streptococcus Gr A* (GAS) was done by Gram stain (Gram positive cocci arranged in chains), colony morphology, β haemolysis, bacitracin sensitivity (0.04 units/ disc), cotrimoxazole resistivity, catalase negativity and PYR positivity, acid production from lactose(+),mannitol(+/-) and ribose(-). Grouping was done by agglutination test using SLIDEX StreptoPlus (BioMerieux India Pvt. Ltd).

Identification of Gr C, G was done by Gram stain from colony, wide β haemolysis, bacitracin and cotrimoxazole sensitivity, catalase, CAMP and PYR negativity, acid production from lactose (+/-) and from ribose (+). Grouping was done by agglutination test using SLIDEX StreptoPlus (BioMerieux India Pvt. Ltd). The numbers of colonies were counted and colonies > 10 were considered to be the causative agents of sore throat.

Antimicrobial susceptibility testing was performed using Disk diffusion by Kirby-Bauer Method and interpreted as per CLSI guideline.

Results

Of the 520 throat swabs collected from patients having sore throat on the day of the visit, 58 (11.15%) were culture positive for group A streptococcus and 10 (1.92%) were culture positive for streptococcus group C,G.

Table 1: The socio-environmental parameters of the study population having sore throat

Socio-environmental parameters	No. of study populations having sore throat	No. of study populations having sore throat due to GAS (n=58)
Area per person (m^2) < 2.33 2.33 to < 3.10 3.10 to <3.72 3.72 to < 4.65 > 4.65	 63 (12.12%) 109 (20.96%) 88 (16.92%) 78 (15%) 182 (35%)	 04 (6.9%) 12 (20.69%) 05 (8.63 %) 02 (3.44%) 35 (60.34 %)
Living in (house) Soil made Brick house Un-finished brick house	 78 (15%) 250(48.07%) 192 (36.93%)	 11 (18.96 %) 33 (56.9%) 14 (24.14 %)
Type of place living in Narrow Open airy	 296 (56.93%) 224 (43.07%)	 37(63.79%) 21(36.21%)
Bathroom Yes No	 400 (76.92%) 120 (23.08%)	 42 (72.41%) 16 (27.59%)
Place of kitchen Separate Open space Within living room	 235(45.20%) 130 (25%) 155 (29.80%)	 03 (5.17 %) 22 (37.93 %) 33 (56.9 %)
Cooking using Gas Indigenous method/ Chullah	 10 (1.92%) 510 (98.08%)	 07 (12.07%) 51 (87.93%)
Tobacco smoking in the house Yes No	 396 (76.15%) 124 (23.85%)	 51 (87.9%) 07 (12%)

Table 1 shows the socio-environmental data of the study population. Regarding the housing conditions of the community 48.07% were living in brick house and separate kitchen facilities were available in 50.96% of study populations. Most families lived in overcrowded homes, with an average of 2.5 persons per room. Households with environmental factors present, e.g. indoor air pollution arising from using chullah for cooking purpose and from there being a tobacco smoker in the family, exhibited a significantly higher incidence of sore throat (P <0.01).

Table 2. Age and Sex-wise distribution of throat isolates

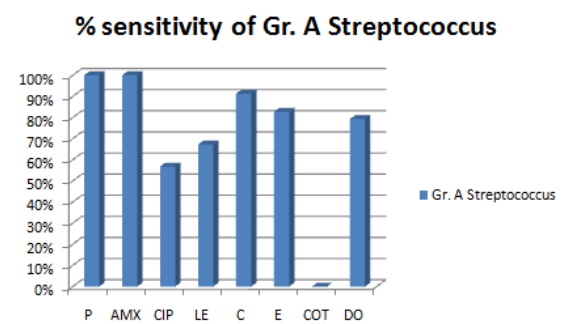
AGE GROUP(in yrs)	Group A Streptococci			Group C G Streptococci		
	Male	Female	Total	Male	Female	Total
<5	05	03	08 (13.79%)	00	00	00 (0%)
5-15	09	13	22 (37.94%)	03	01	04 (40%)
16-25	06	10	16 (27.59%)	02	02	04 (40%)
26-35	06	06	12 (20.68%)	01	01	02 (20%)

Incidence of GAS sore throat among males were 44.8% and among females were 55.2% whereas incidence of sore throat due to Group C G among males were 60% and among females were 40%. So there was no significant difference in the incidence of sore throat or GAS sore throat between males and females. The incidence of sore throat varied according to age (P <0.01), being higher among the 5–15-years age group (Table 2).

Table 3: Seasonal variation of sore throat over one year study period

	Total culture positive sore throat	GAS sore throat	CG sore throat
September to November (Autumn)	14 (20.59%)	12 (17.64%)	02 (2.95%)
December to February (Winter)	42 (61.77%)	36 (52.95%)	06 (8.82%)
March to May (Summer)	06 (8.82%)	06 (8.82%)	-
June to August (Rainy season)	06 (8.82%)	04 (5.87%)	02 (2.95%)

The incidence was highest during the winter months (P 0.01).Table4: Antibiotic susceptibility of GAS isolated from throat swabs



P: Penicillin (10µg), AMX:Amoxicillin (10µg), COT: Cotrimoxazole (1.25/23.75µg), E: Erythromycin (15µg), CIP: Ciprofloxacin (5µg), DO: Doxycycline (30 µg), C: Chloramphenicol (30 µg)

Discussion

In India isolation rates of GAS in children with pharyngitis have ranged from 4.2% to 13.7% (2, 3) which is comparable to the rates reported from developed countries. However, in closed and crowded communities the rates of isolation and spread of GAS infection may be much higher. In our study the isolation rate of GAS from throat swab culture was 11.15%. The prevalence of asymptomatic carriage of GAS in different parts of India has been reported to lie in the range of 11.2–34% (4). The point prevalence of β-haemolytic streptococcal sore throat was 13.6% in a rural area of Varanasi, India (5). In southern India, 12% of all pharyngitis cases were caused by GAS (3). In school-aged children in Delhi, the prevalence of GAS pharyngitis was 13.7% (4).

The incidence of GAS sore throat was highest in winter months in our study. During winter, children mostly live indoors in

crowded conditions, which may increase the transmission of infection. In rural Egypt, the highest streptococcal carrier rate was observed in late autumn and early winter and the lowest rate in the summer months (6). In an Indian community near Varanasi the highest point prevalence was seen in winter (5), and in Europe the highest incidence was found in autumn (7). Regarding age, the peak incidence of GAS pharyngitis was in children aged 5–15 years (8). The reason may be they move to school, thus being exposed to infection from other strains of streptococci.

The incidence of sore throat as well as GAS sore throat was not significantly different between males and females. While some studies have reported that the prevalence of b-haemolytic streptococcal pharyngitis was higher among females than among males in Delhi and other places in India (9, 10) others have reported that GAS pharyngitis did not vary according to sex (5).

In the present study, while socioeconomic status had no influence on the incidence of sore throat, environmental factors such as tobacco smoking in the family and use of chullah (indigenous method) had an influence on GAS infection.

Most children in the study population were living in overcrowded conditions, with an average of 2.5 persons per room.

Regarding antimicrobial sensitivity, macrolides including erythromycin and clindamycin have been widely used for treatment of acute pharyngitis and invasive infection of GAS respectively. Kim et al (11) recently reported a high frequency of resistance to erythromycin in GAS, particularly in countries where antibiotics are overused. Of all throat isolates, 95.0%were predominantly resistant to erythromycin, 70.0% to clindamycin, 56.0% to azithromycin and 24.0% to clarithromycin according to the study done in Sindh.(12) Tamayo et al (13) reported the erythromycin resistance rate to be 21.7% in the study done in Spain in 2004. Ciftci et al (14) reported resistance to erythromycin,clarithromycin, azithromycin and clindamycin as 3.8%, 5.2%, 4.2% and 3.0% respectively. Alberti et al (15) reported increased resistance of S. pyogenes to ciprofloxacin in Spain at the highest rate ever published and it is 63.3%. Of all the isolates analyzed in our study, 17.3% were resistant to erythromycin, 20.7% to doxycycline and 43.2% to ciprofloxacin. All the isolates were sensitive to beta lactam antibiotics (penicillin and amoxycillin). A study done in France by Binjen et al (16) found all isolates of *S. pyogenes* were susceptible to amoxycillin. The results of our preliminary study highlights the importance of regular surveillance programs to monitor the rate of GAS carriage and the antibiotic susceptibility of GAS isolates in the community as it shows reduced antibiotic susceptibility even in rural areas .

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