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Archives of Applied Science Research, 2015, 7 (1):15-21
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-508X
CODEN (USA) AASRC9

Epidemiology and chemotherapy of newly emerging pathogens from beta hemolytic Streptococcal Group B, C and G

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ABSTRACT

Resistance to antibiotics has been noted in all species of streptococci however it varies from country to country. The goal of the present study was to get the knowledge about the epidemiology and chemotherapy of Streptococcal Group B, C and G as emerging pathogens. In this study 28 beta hemolytic streptococci were isolated from various clinical specimens from the hospitals and pathological laboratories of Karachi, Pakistan from November 2013 to October 2014. Among the collected strains we identified 18 (64%) GBS, 8 (29%) GGS and 2 (7%) GCS. Most of the GBS and GGS strains were isolated during the rainy season of the year and the majority of the strains were collected from throat swabs. Kibry-Bauer disc diffusion method and MIC analysis indicated that the strains of GGS were comparatively more resistant to various antibiotics as compared to GBS. GGS strains were 50% resistant to most macrolide antibiotics. Furthermore, these strains were also 39% resistant to clindamycin and 25% to penicillin, cefotaxime and ciprofloxacin. Among GBS the highest rate of resistance was observed against clarithromycin (43%), followed by azithromycin, erythromycin and clindamycin (39%). GCS strains were sensitive to all the antibiotics except being 50% clindamycin resistant. However all the strains in this study were sensitive to augmentin antibiotic. MIC analysis indicated that both GBS and GGS strains had the highest MIC against clindamycin. The findings of the present study would provide awareness about the epidemiology and chemotherapy of group B, C and G as the emerging pathogens among from our region.

Key words: Prevalence, GCS, GGS, GBS, Pathogen, drug resistance, *Streptococci*

INTRODUCTION

The β-hemolytic pattern of Streptococci was first described by J. H. Brown while Rebecca Lancefield differentiated β-hemolytic streptococci on the basis of their cell wall antigens. These antigens are group-specific and are either polysaccharides (as in group A, B, C, E, F and G) or teichoic acid (as in group D and N) or lipoteichoic acid (as in group H) [1]. The genus *Enterococcus* was separated from the genus *Streptococcus* in 1984 on the basis of DNA-DNA and DNA-RNA hybridization studies [2]. Although β-hemolytic property is variable among the members of *Enterococcus*, identification of Lancefield group antigen together with phenotypic characteristics add in the differentiation from other β-hemolytic streptococci [3].

Group B Streptococci (GBS) are the significant cause of morbidity and mortality in newborns, in children younger than 3 months and in pregnant and non-pregnant women [4,5]. Specially this group is responsible for early-onset and late-onset diseases in children, infections in amniotic fluid of pregnant women and intrauterine infections [6,7]. The mortality rate of early onset sepsis has estimated about 50% [8].

Group C and G Streptococci are usually considered as emerging nosocomial and opportunistic pathogens [9]. These pathogens are reported to cause a spectrum of human diseases like pharyngitis, cellulitis, endocarditis, meningitis, arthritis, osteomyelitis, skin and wound infections, urosepsis and pneumonia [9,10]. Furthermore, immunocompromised patients or patients with underlying medical problems such as diabetes mellitus, cardiovascular diseases, malignancy, liver or renal dysfunction are at risk of getting group C and group G infections [11,9].

In the last decade many reports have been published by the developed and developing countries indicating the emergence of macrolide resistance among β-hemolytic streptococci [12,13,14]. The significance of streptococcal studies has increased due to the appearance of multidrug resistant strains and especially high level resistance against aminoglycosides and vancomycin [15].

The purpose of the present study is to document and report the epidemiology of newly emerging beta hemolytic streptococcal infections from Karachi, Pakistan. The current project aims at determining the antibiotic resistance pattern and the minimum inhibitory concentration (MIC) of commonly used antibiotics among indigenous clinical emerging streptococcal pathogens. Thus the goal of the proposed study is to get knowledge about the epidemiology and chemotherapy of Streptococcal Group B, C and G as emerging pathogens. To our knowledge no data is available about Group C and G strains from Pakistan thus this would be the first study reporting such strains from Pakistan.

MATERIALS AND METHODS

(1) Collection of beta hemolytic streptococcal strains: Twenty eight beta hemolytic strains were collected from different pathological laboratories and hospitals of Karachi.

(2) Identification of beta hemolytic streptococcal strains: All of the collected strains were purified (to get the pure culture) and identified by routine diagnostic tests like catalase test, Beta hemolysis.

(3) Grouping of beta hemolytic streptococcal strains: In order to group beta hemolytic streptococcal strains into Group B, C and G, **a**ll of the collected strains were grouped by Lancefield grouping kit (Oxoid).

(4) Antibiotic resistance pattern: The antibiotic resistance pattern of collected group B, C and G streptococcal strains were checked by commonly prescribed antibiotics like Erythromycin, Clindamycin, Azithromycin, Penicillin, Ciprofloxacin, Cefotaxime, and Clarithromycin [16].

(5) Determination of MIC: Determination of minimum inhibitory concentration (MIC) was done by broth dilution method [17].

RESULTS

In this study 28 beta hemolytic streptococci were isolated from various clinical specimens from the various hospitals and pathological laboratories of Karachi, Pakistan from November 2013 to October 2014. Among the collected 28 strains we identified 18 (64%) GBS, 8 (29%) GGS and 2 (7%) GCS (figure 1).

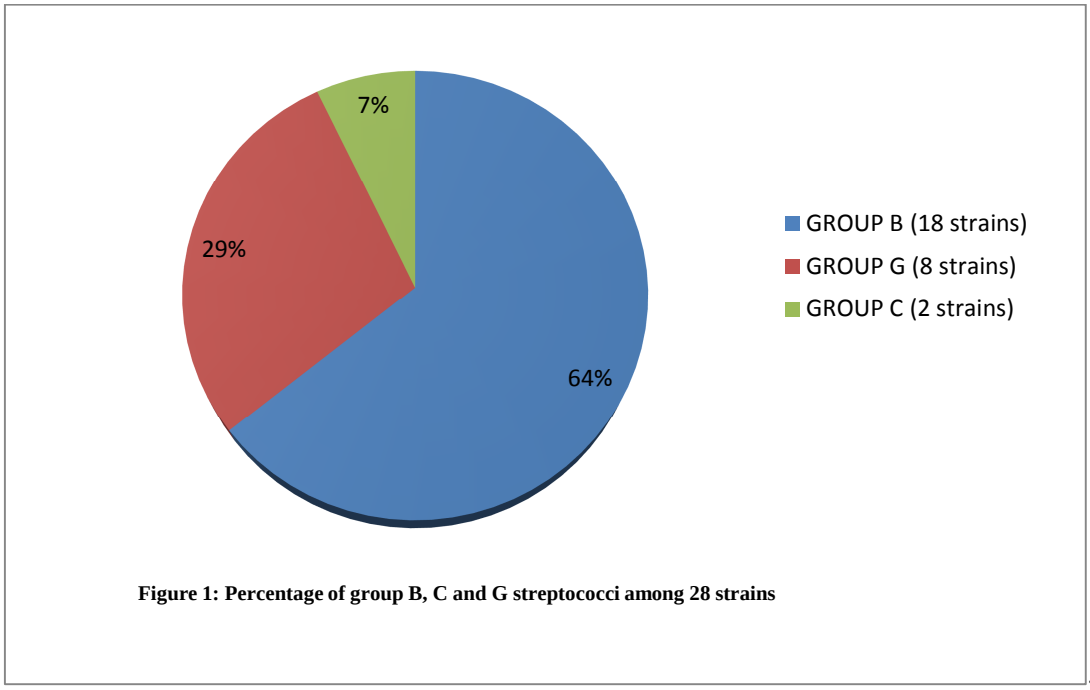

Figure 1: Percentage of group B, C and G streptococci among 28 strains

Most of the GBS and GGS strains were isolated during the rainy season of the year that is from July to October (figure 2).

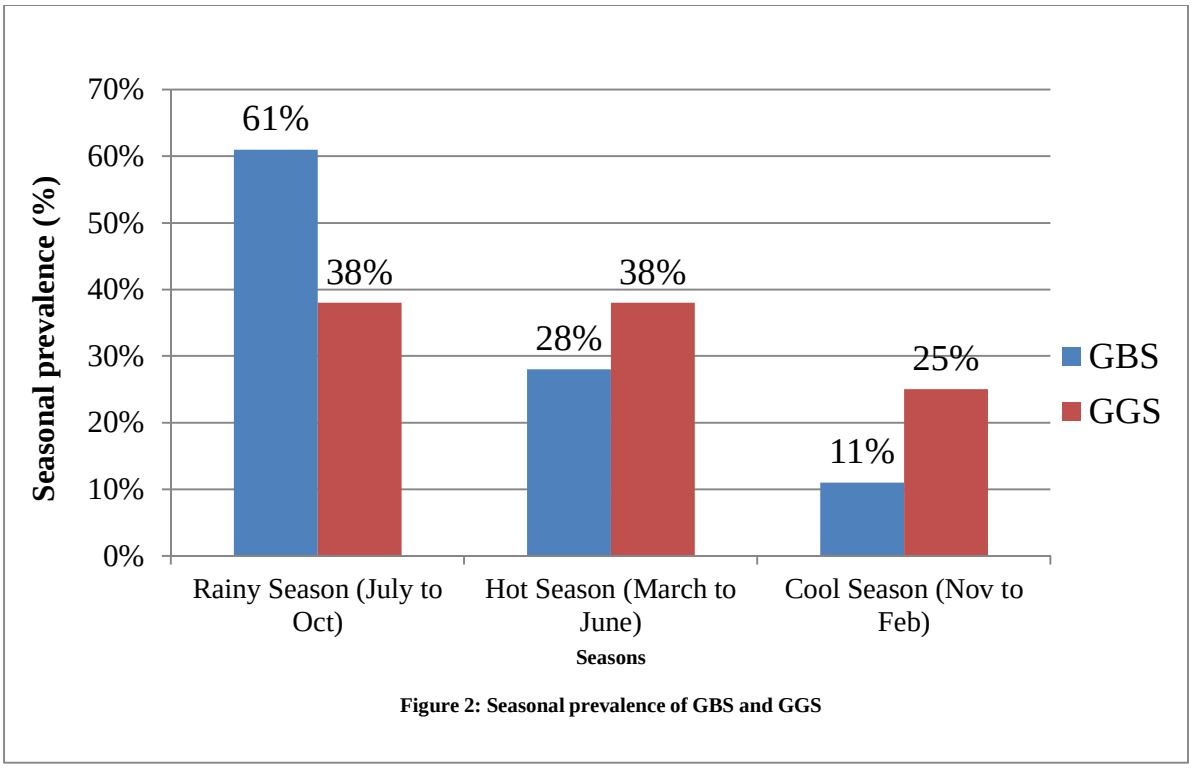

Figure 2: Seasonal prevalence of GBS and GGS

Most of the GBS strains were collected from throat swabs 7 (39%), followed by urine and pus 4 (22%). While only one strain was isolated each from blood, wound and vaginal swab (figure 3).

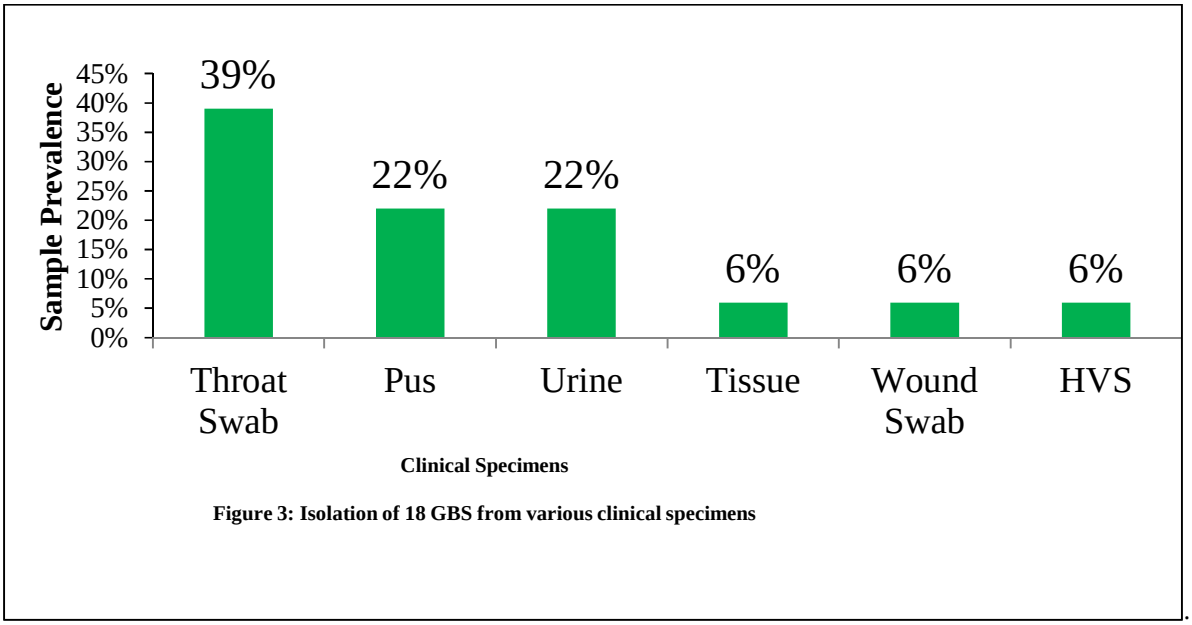

Figure 3: Isolation of 18 GBS from various clinical specimens

In case of GGS strains 6 (75%) were collected from throat swab, while 1 strain each from tissue and wound swab. Both strains of GCS were isolated from throat swabs.

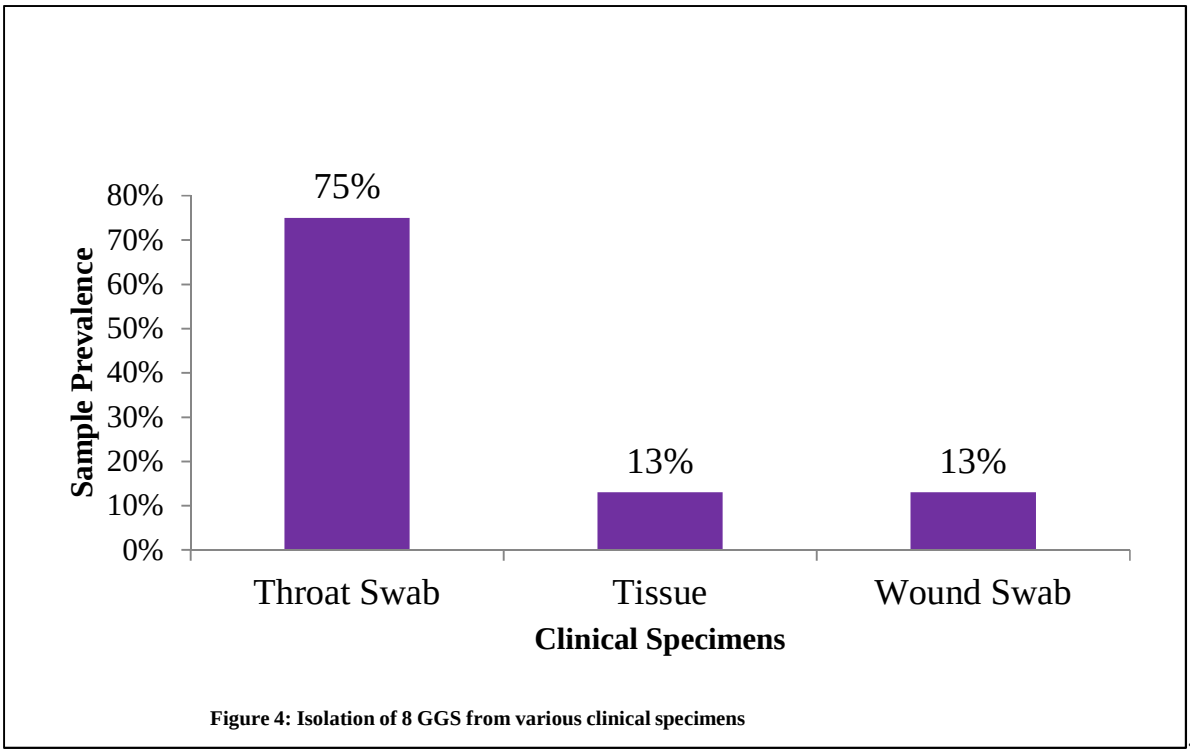

Figure 4: Isolation of 8 GGS from various clinical specimens

Over all the strains of GGS were comparatively more resistant to various antibiotics as compared to the strains of GBS. Among GGS strains the highest rate of resistance was seen against macrolide group that is 50% against azithromycin, erythromycin and clarithromycin each, followed by 39% against clindamycin and 25% against penicillin, cefotaxime and ciprofloxacin (figure 5). Among GBS the highest rate of resistance was observed against clarithromycin 43%, followed by 39% resistance against azithromycin, erythromycin and clindamycin. 18%

resistance was against cefotaxime and 10% resistance was found against penicillin and ciprofloxacin. GCS strains were sensitive to all the antibiotics except clindamycin which showed 50% resistance rate. However all the strains in this study were sensitive to augmentin antibiotic (figure 5).

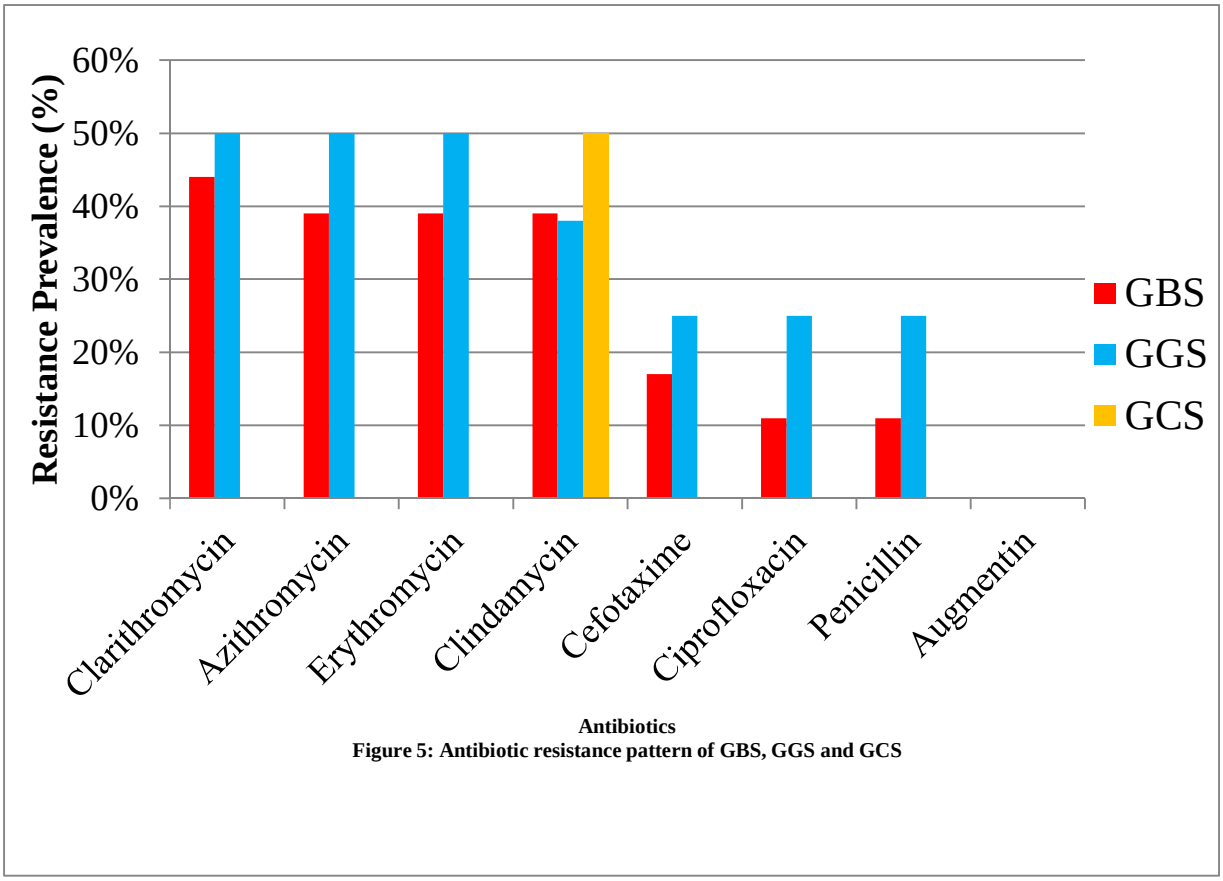

Figure 5: Antibiotic resistance pattern of GBS, GGS and GCS

The experiment of MIC revealed that both GBS and GGS strains had the highest MIC against clindamycin. MIC analysis also confirmed that GGS strains have much higher antibiotic resistance level as compared to the GBS strains (table 1 and 2).

Table-1. Minimum Inhibitory Concentration of GBS

Antibiotic Group	Antibiotics (No. of Strains)	Breakpoints (Sensitive/ Resistance)	Range (µg/ml)	MIC (µg/ml)	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)	Level of resistance (No. of times)
Macrolides	**Clarithromycin** (8)	≤0.25/≥1	0.1 - 32	8	28	8	8
	Azithromycin (7)	≤0.5/≥2	0.5- 1024	512	128	256	256
Lincosamide	**Clindamycin** (7)	≤0.25/≥1	0.1- 1024	512	64	128	512
Quinolone	**Ciprofloxacin** (2)	≤1/≥4	0.5 - 64	32	8	32	8
MIC break points are according to CLSI							

Table-2. Minimum Inhibitory Concentration of GGS

Antibiotic Group	Antibiotics (No. of Strains)	Breakpoints (Sensitive/ Resistance)	Range (µg/ml)	MIC (µg/ml)	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)	Level of resistance (No. of times)
Macrolides	**Clarithromycin** (4)	≤0.25/≥1	2 - 1024	512	128	256	512
	Azithromycin (4)	≤0.5/≥2	1 - 1024	1024	256	512	512
Lincosamide	**Clindamycin** (3)	≤0.25/≥1	1 - 1024	1024	512	512	1024
Quinolone	**Ciprofloxacin** (2)	≤1/≥4	2 - 1024	256	64	256	64

MIC break points are according to CLSI

DISCUSSION

Over the last years, the emergence of resistance to antibiotics has complicated the management of streptococcal infections. The resistance of streptococci to antibiotics is increasing and seems to be linked to the consumption of antibiotics. Resistance to antibiotics have been noted in all species of streptococci however varies from species to species and according to countries. Infections of β-hemolytic streptococci are classically treated with penicillin, whereas, macrolides or lincosamides are recommended as the alternative antibiotics if patient is allergic to penicillin [18,19].

Currently although no *Streptococcus pyogenes* has shown resistance to betalactams, this phenomenon has been reported for some strains in the B, C, and G groups and 56 % among oral streptococci. The resistance to macrolides has been reported as 41 % for *Streptococcus pyogenes*, 46 % for group B streptococci and 63 % for oral streptococci responsible for severe infections [20]. This evolution of drug resistance is a matter of great concern for the chemotherapy and in the management of streptococcal diseases.

Streptococcus agalactiae is the only significant member of GBS causing infection in women and newborns. It causes pneumonia, bacteremia and meningitis in newborns and colonizes in female reproductive tracts, increasing the risk for premature rupture of membranes during pregnancy, and transmission to the infants causing sepsis in neonates [5]. According to our results GBS were 50% resistant against azithromycin, erythromycin and clarithromycin each, while 39% against clindamycin and 25% against penicillin, cefotaxime and ciprofloxacin. However according to a recent study done in Malaysia the resistance rates for erythromycin was 23.3%, clindamycin 17.5% and tetracycline 71.8 % [21].

GGS and GCS are emerging pathogens, however their potential to cause infections is not fully recognized yet. Recently GGS has also been reported to cause brain abscess [22]. According the present study upto 50% resistance has been noted among GGS against macrolide drugs. Macrolide resistance has also been reported from several countries among GGS for example in hong kong it is reported to be 24% and 19% from USA [23]. Another recent study has reported the resistance among different hospitals of Taiwan varied from 15-45.5% for erythromycin and from 7-36.4% for clindamycin [24].

The results of the current study would help us in getting knowledge about current resistance pattern of these strains against commonly prescribed antibiotics. This would discourage rational use of antibiotics in treating community acquired infections and avoiding emergence of drug resistance. This study would further help us in better understanding streptococcal group B, C and G epidemiology and chemotherapy. The knowledge about these emerging pathogens would also alert and aware doctors, para-medical staff and patients to better deal with the streptococcal infections in our community.

Acknowledgement
This research project was funded by Dean Faculty of Science, University of Karachi research grant DFS/2014 given to Dr Zaid Ahmed Pirzada.

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