Response of Different Antibiotic Resistant Group of *Streptococcus pyogenes* to Environmental Stresses

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Abstract *Streptococcus* species is considered as an important pathogen for human and animals. The antibiotic resistance mechanism in this species is continuously increased. On the other side, the tolerance of environmental stresses play an effective role in the severity of many streptococcal causative disease. In this study we assayed survey on the causative agents of pharyngitis and tonsillitis patients. The predominant causative strain was *Streptococcus pyogenes* with 93 % isolating ratio frequency. The other pathogenic species were *S. agalactia* 5.3 % and *S. pneumonia* 1.7 %. According to the antibiotic resistant test the *S. pyogenes* isolates were classified into six different groups. A selected strain from each antibiotic resistant group was tested for tolerance of a restrictive environmental factors. The variations of the environmental niches of isolates were in consistence with their antibiotic resistant variation.

Keywords *Streptococcus pyogenes* · Antibiotic resistant · Environmental stresses

Introduction

Group A *Streptococcus* (GAS) is a gram-positive pathogen that causes many infections (pharyngitis, septicemia, toxic

shock, and necrotizing fasciitis) and post-infectious sequelae (rheumatic heart disease and glomerulonephritis). Humans are the natural host and sole reservoir of GAS. The organism can survive and replicate in diverse anatomic sites such as the skin, throat, female urogenital tract, lower gastrointestinal tract, and blood [1]. The same strain may cause a more serious, invasive disease in another patient. The more severe diseases are caused by the invasion of GAS into deeper tissues and include necrotizing fasciitis, septicemia, and streptococcal toxic shock syndrome, all of which can be life threatening. For GAS to cause disease in such diverse host tissues, this organism must be able to withstand a variety of environmental factors and grow in different microenvironments. GAS can face many stress conditions within the human host, such as the increased salt concentration on the skin, the low pH of the vagina, and the pH fluctuations within the nasopharynx. Also, the bacteria must be able to overcome the stress conditions that arise as a consequence of the GAS infection itself, such as high temperature resulting from fever and decreased pH as a result of abscess formation. Bacteria often sense and respond to specific environmental changes by using two-component signal transduction regulatory systems [2, 3]. The terminal target for this system are DNA binding proteins CovR and CovS which has been assumed that they have cotranscribed effects [4]. The response difference for environmental stresses between *S. pyogenes* could be used as well as other markers to detect the intra specific differences.

Approximately 20 years ago, the virulence associated with *S. pyogenes* increased worldwide. Changes in the predominate serotypes in natural populations and in the repertoire of genes encoding exoproteins, including superantigens, are thought to have contributed to the increase in virulence [5, 6].

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Although large-colonyforming-hemolytic streptococci (LCF-BHS) are still susceptible to β -lactams, macrolides or lincosamides are recommended as alternative choices when indicated [7–9]. However, recent studies have shown that changes in the susceptibility of LCF-BHS to erythromycin and clindamycin have been substantially decreased, although differences in resistance rates to these agents exist according to geographical variation and investigators [10–12].

The objectives of the present study is to investigate the incidence and trend in susceptibility among the streptococci isolated from clinical specimens in Minufiya region and to clarify the microbial response of different antibiotic resistance groups against different environmental stresses.

Materials and Methods

Isolates of group A *Streptococcus* recovered from throat swab specimens (one per patient) adults and children with streptococcal pharyngitis, secondary to treatment failure. The specimens were cultured immediately on blood nutrient agar. The bacterial isolates were selected, categorized into several groups of Streptococci according to Bergey's (1983) [13]. Streptokinase specific activity of the isolates were tested using both animal and human plasminogen on agar plates according to Silverstein (1975) [14]. Different antibiotic discs were used to determine the antibiotic resistance of *S. pyogenes* isolates [15]. For all experiments, the *S. pyogenes* ATCC 19615 strain was simultaneously tested as a reference strain. The following antimicrobial agents were obtained from (Oxoid UK) amoxicillin clavulanic acid (AMC30), cefadroxil (CFR30), cephradine (CE30), ciprofloxacin (CIP5), erythromycin (E15), norfloxacin (NOR10), piperacillin (PRL), rifamycin (RF30), streptomycin (S10), sulbactam ampicillin (SAM 20).

The heart infusion media was used for experiments of antibiotic resistance and growth optimization. The bacterial isolates were cultured on solid medium supplemented antibiotic discs and incubated at 37 °C for 24 h. The growth parameter was measured using the optical density of the bacterial culture at 600 nm.

Results

Characterization of Bacterial Isolates

Forty-five infected samples were isolated from pharyngitis and tonsillitis patients. The microbiological analysis of the pathogenic agents was demonstrated in (Table 1), the predominant causative strain was *Streptococcus pyogenes*

with 93 % isolating ratio frequency. The other pathogenic species were *S. agalactia* 5.3 % and *S. pneumonia* 1.7 %.

Specific Streptokinase Production

The specific streptokinase secretion of the characterized strains were detected using agar plates with addition of human or animal plasminogen. All *S. pyogenes* isolates show active reaction with human plasminogen while all other isolates of *S. pneumonia*, *S. agalactia* showed a comparable activities with animal plasminogen. The enzyme activity was demonstrated as a clear zone formation as it was demonstrated in Fig. 1.

Antibiotic Resistance

According to the antibiotic resistant test the *S. pyogenes* isolates were classified into six different groups. All bacterial isolates were resistance to streptomycin and sensitive to cefadroxil and piperacillin. The conventional streptococcal antibiotic erythromycin was effective in case of standard strain while some bacterial isolates (four of six) show resistance to erythromycin. The sensitivity to cephradine was common between the standard strain and some isolated bacterial isolates. Comparing to standard strain some isolates of *S. pyogenes* demonstrated higher resistance to other antibiotic like amoxicillin clavulanic acid (AM), norfloxacin (NOR) and sulbactam ampicillin (SAM). Most of local isolated streptococcal strains are resistant to erythromycin (E15). From these data it can be concluded that the most potent antibiotic was cefadroxil (CFR) and streptomycin (S) was the most compromised one (Table 2).

Tolerance for Environmental Stresses

Different media were tested for best growth yield of *S. pyogenes*. The best medium for bacterial growth was brain heart infusion media at 37 °C. This medium was used to determine the tolerance limit of bacterial isolates at different environmental stresses. The antibiotic resistant groups showed similar growth patterns at different temperatures 15, 20, 30, and 35 °C. The optimum growth was attained at 35 °C for all groups (Table 3). However, the antibiotic resistant groups 2, 3 showed better heat tolerance at 40 °C. The effect of pH degrees on growth curve showed optimum growth at pH 7. At other pH values the growth pattern of the different groups were similar. The different response was occurred only at pH 8 (Table 4). The NaCl gradient effect showed very similar response for the different bacterial isolates (Table 5) however, the antibiotic resistant group 3 was the only one that demonstrate higher

Table 1 The effect of some antibiotics on the growth of different strains of streptococci

Exp	Strains					
	<i>S. agalactia</i>	<i>S. dysgalactia</i>	<i>S. pyogenes</i> (standard strain)	<i>S. pneumonia</i>	<i>S. bovis</i>	<i>S. pyogenes</i>
Gram reaction	+	+	+	+	+	+
Growth in air	+	+	+	+	+	+
Catalase	–	–	–	–	–	–
Growth in air plus 5 % CO ₂	+	+	+	+	+	+
Growth anaerobically	+	+	+	+	+	+
Growth at °C						
10	d	–	–	–	–	–
45	–	–	–	–	d	–
Growth at pH 9.6	–	–	–	–	d	–
Growth with						
6.5 % NaCl	d	–	–	–	–	–
40 % bile	d	–	–	–	+	–
0.25 % optocin	+	+	+	+	+	+
Alpha-hemolysis	–	+	–	+	dw	–
Beta-hemolysis	d	–	+	–	–	+
Hydrolysis of						
Arginine	+	+	+	+	–	+
Hippurate	+	–	–	–	–	–
Esculin	–	–	–	–	–	d
Acid from						
Inulin	–	–	–	–	–	d
Lactose	d	+	+	+	+	+
Mannitol	–	–	–	–	d	–
Raffinose	–	–	–	+	Nd	+
Ribose	+	Nd	–	–	–	–
Salicin	d	d	+	+	+	–
Sorbitol	–	d	–	–	d	–
Trehalose	+	+	+	+	d	+
Production of						
Alkaline phosphatase	+	+	+	–	–	–
α -Glucosidase	–	–	–	–	d	–
β -Glucuronidase	d	+	–	–	–	–
β -Glactosidase	–	–	–	–	–	d
Pyrrolidonearylamidase	–	–	+	+	–	d
Voges-proskauer test	+	–	–	–	Nd	–
Lancefield serological group	B	C	A	A	D	None

tendency for salt tolerance at 6 % NaCl comparing the other groups.

Discussion

Comparing to the standard strain the local isolated streptococci in our study show high resistance ratio against erythromycin as well as other antibiotics like amoxicillin

clavulanic acid, norfloxacin and sulbactam ampicillin. Until the 1980s, pathogenic strains of *S. pyogenes* were generally considered uniformly susceptible to erythromycin and clindamycin, but resistance spread rapidly in the 1990s. The prevalence of erythromycin resistant *S. pyogenes* has been reported to be variable and depends on the country, selective pressure, serogroup, serotype, age, and season, [16]. High rates of erythromycin-resistance have been reported in San Francisco (27 %) and Genoa (50 %). Only

Fig. 1 Comparison of streptokinase secretion activity of different isolates of streptococci. **a** The human plasminogene assay, (11) is the clear zone of *S. Pyogenes* standard strain. **b** The animal plasminogene assay

Table 2 The effect of some antibiotics on the growth of different strains of streptococci

St. groups	Antibiotics									
	PRL ₁₀₀	AMC ₃₀	NOR ₁₀	SAM ₂₀	RF ₃₀	CE ₃₀	CiP ₅	E ₁₅	CFR ₃₀	S ₁₀
1	S	S	S	S	S	S	S	S	S	R
2	S	S	R	S	S	S	S	R	S	R
3	S	S	R	S	S	S	R	R	S	R
4	S	S	R	S	S	R	R	S	S	R
5	S	S	S	R	S	R	R	R	S	R
6	S	R	R	R	R	R	S	R	S	R
Standard	S	S	S	S	S	R	S	S	S	R

Table 3 The effect of different temperatures on the growth of different strains of streptococcus grown on liquid medium

Strains	Temperature					
	15 °C	20 °C	25 °C	30 °C	35 °C	40 °C
1	0.13	0.32	0.73	0.92	1.38	0.30
2	0.14	0.28	0.81	1.12	1.56	0.65
3	0.21	0.31	0.82	0.94	1.72	0.80
4	0.14	0.32	0.85	1.02	1.53	0.07
5	0.13	0.27	0.72	1.05	1.26	0.06
6	0.21	0.35	0.83	1.15	1.27	0.03
Standard	0.20	0.35	0.74	1.02	1.30	0.35

Data are expressed as O.D₍₆₀₀₎

Table 4 The effect of different pH degrees on the growth of different antibiotic groups of *S. pyogenes*

Strains	pH degrees				
	5.0	6.0	7.0	8.0	9.0
1	0.08	0.85	1.26	0.90	0.04
2	0.08	0.55	1.03	1.25	0.05
3	0.09	0.71	1.33	0.61	0.03
4	0.09	0.67	1.23	0.51	0.02
5	0.08	0.53	1.25	0.89	0.02
6	0.09	0.62	1.12	1.01	0.01
Standard	0.07	0.73	1.30	0.62	0.06

16.2 % of GAS strains, 28.3 % of GGS strains and 25 % of GCS strains were erythromycin resistant [17].

One mechanism of antibiotic resistance in *S. pyogenes* is the Macrolide efflux, which is effected by a membrane protein encoded by the *mef* class genes [18, 19]. This antibiotic resistance mechanism has recently emerged among *Streptococcus pyogenes* and *Streptococcus pneumoniae* in

many countries [20]. These results demonstrate clearly that antibiotic resistance could be used as evidence for genetic changes and as a tool for interspecific grouping in bacteria.

The continued high resistance rates to erythromycin, clindamycin, and tetracycline are considered related to the clonal spread of serotype V with a multi-drug resistance phenotype [21]. The classification of *S. pyogenes* as a serotype groups was used to detect and differentiate the virulence of infection and the previous results indicate that it

Table 5 The effect of different concentrations of NaCl on the growth of different strains of *streptococcus* grown on liquid medium

Strains	NaCl %							
	0.0	0.5	1	2	3	4	5	6
1	1.02	1.50	1.12	0.81	0.53	0.32	0.17	0.05
2	0.99	1.37	1.15	1.00	0.56	0.32	0.21	0.02
3	0.88	1.60	1.20	1.00	0.80	0.51	0.31	0.13
4	1.03	1.24	1.05	0.62	0.44	0.20	0.10	0.05
5	0.92	1.40	1.00	0.80	0.51	0.35	0.16	0.01
6	0.97	1.28	1.04	0.71	0.60	0.29	0.15	0.04
Standard	0.98	1.31	0.99	0.81	0.60	0.31	0.20	0.02

Data are expressed as O.D.₍₆₀₀₎

could be an effective way for detection of antibiotic resistance and vice versa. Bacciaglia et al. (2007) [22] demonstrate that the change of antibiotic resistance pattern was related to genetically reforming of the bacterial genome. On the other side, the bacterial biochemical characterization is also closely related to the genetic make up and any change in these activities could be a strong evidence about genetic change. To demonstrate the relation between change of antibiotic resistance pattern and change of other bacterial biochemical characters, several environmental stresses as well as nutrition requirements were tested. However, the relation between genetic elements that control the metabolic activities and expression of virulence factors has been proved [23–25]. Other genetic factor, CovS plays essential role for resistance against low pH, high temperature and high osmolarity in *S. pyogenes* [4]. The high growth temperature was associated with 2.4 fold increase in the transcription of virulence factor streptolysin O in the aggregate. The results demonstrate that transcription of genes encoding iron transporters and iron-dependent regulators are similarly affected at decreased (29 vs. 37 °C) and increased (40 vs. 37 °C) temperatures. One explanation for this pattern of gene regulation is that growth at 29 and 40 °C. [26].

In this study some antibiotic resistance groups of *S. pyogenes* isolates show a response difference for different environmental stress, specially concerning the pH and salinity stress. This can be further evidence about the genetically change of the local isolated streptococcal strains.

All streptococcal isolated strains rather than *S. pyogenes* secrete streptokinase with animal plasmin binding property. This means that the infection may was resulted as animal carrier.

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