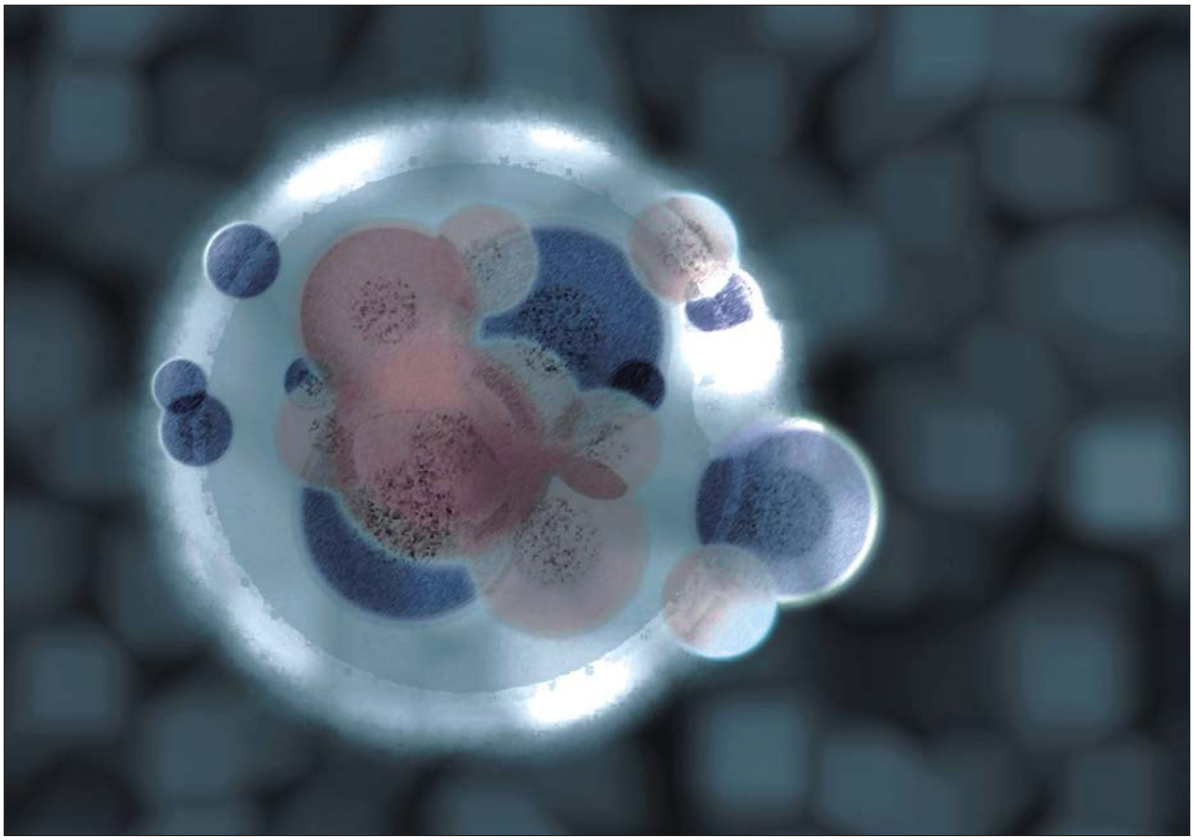

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Characterization of β-hemolytic *Streptococcus* Strains Isolated from Patients of Severe Invasive Streptococcal Infections

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Abstract: *Streptococcus pyogenes* (group A *streptococcus*) is one of the most common human pathogens. It causes a wide array of infections, the most frequent of which is acute pharyngitis (strep throat). From the late 1980s, Streptococcal Toxic Shock-like Syndrome (STSS) caused by *S. pyogenes* became a serious problem in both developed and developing countries. Symptoms such as pharyngitis, fever and pain may suddenly develop and the disease may progress very rapidly in some patients to soft tissue necrosis, acute kidney failure, Adult Respiratory Distress Syndrome (ARDS), Disseminated Intravascular Coagulopathy (DIC) and Multiorgan Failure (MOF), leading to shock and death. In this review, characterization of genotypes and antimicrobial susceptibility of strains isolated from patients of severe invasive streptococcal infections are summarized.

Key words: Severe invasive streptococcal infections, typing, antimicrobial susceptibility

SEVERE INVASIVE STREPTOCOCCAL INFECTIONS

Streptococcus pyogenes (group A *Streptococcus*) is one of the most common human pathogens. It causes a wide array of infections, the most frequent of which is acute pharyngitis (strep throat) and impetigo (pyoderma)[1]. From the late 1980s, Streptococcal Toxic Shock-like Syndrome (STSS) caused by *S. pyogenes* became a serious problem in many countries[2-9]. Symptoms such as pharyngitis, fever and pain may suddenly develop and the disease may progress very rapidly in some patients to soft tissue necrosis, acute kidney failure, Adult Respiratory Distress Syndrome (ARDS), Disseminated Intravascular Coagulopathy (DIC) and Multiorgan Failure (MOF), leading to shock and death. The diagnostic standard was proposed from Centers for Diseases Control and Prevention (CDC)[10].

VIRULENCE FACTORS

It is clear that this disease is caused by the infection of *S. pyogenes*, however the pathogenic mechanism is not clear. *S. pyogenes* strains produce many extracellular products. Streptolysin O and streptolysin S, which have hemolytic activity, are toxic to a variety of human cells *in vitro* and *in vivo*[11-15]. M proteins protect *S. pyogenes* from phagocytosis by polymorphonuclear leukocytes[16,17]. Streptococcal Pyrogenic Exotoxins (SPE), SpeA, SpeB and SpeC, are called scarlatina or erythrogenic toxins. SpeA and SpeC have a superantigen activity and release a large amount of cytokines by stimulating T cells with particular Vβs dependent on MHC-II molecules. Recently, it is reported that SpeF[18,19], SpeG[20], SpeH[20], SpeJ[21], SpeK[22], SpeL[23], SpeM[23], SSA[24] and SmeZ[20,25,26] have also superantigen activities. Some of them are encoded on the genome of bacteriophage. For example, a horizontal transfer of the phage NIH1.1 carrying *speL* was speculated to be involved in the emergence of new *emm3 S. pyogenes* isolates[27]. SpeB has a cystein protease activity and induce apoptosis in macrophages and epithelial cells, possibly through activation of the caspase cascade[28,29]. Hyaluronidase degrades hyaronic acid of tissues. Streptokinase activates plasminogen, induces plasmin and causes degradation of fibrin and fibrinogen, so that it inhibits the cascade of blood coagulation[30]. Streptokinase production is required for induction of poststreptococcal glomerulonephritis in an experimental mouse model[31]. Mac-1 (also known as IdeS) binds to the surface of human polymorphonuclear leukocytes (PMNs) and inhibits opsonophagocytosis and production of reactive oxygen species[32]. ScpA specifically cleaves the complement factor C5a[33], a chemotactic complement factor and inhibits recruitment of phagocytic cells to the infectious site[34]. C5a is also known to activate the function of neutrophils that phagocytize the bacteria, underlining the relevance of the activity of ScpA[35].

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Table 1: Characteristics of erythromycin resistant strains[53]

Strain No.	Isolation year	Antimicrobiol susceptibility[1]			Possession of resistant gene[2]			*emm* allele
		EM[3]	CLDM[3]	TEL[3]	*ermA*	*ermB*	*mefA*	
61	1998	R	S	S	-	-	+	12
125	2000	R	R	R	-	+	-	1
162	2001	R	S	S	-	-	+	1
170	2001	R	S	S	+	-	-	58
184	2001	R	S	S	+	-	-	58
220	2002	R	S	S	-	-	+	1
225	2003	R	R	R	-	+	-	1
237	2003	R	R	R	-	+	-	1

[1]R: resistant, S: Susceptible, [2]+: Positive, -: Negative, [3]EM: Erythromycin, CLDM: Clindamycin, TEL: Telithromycin

EMM GENOTYPES IN ISOLATES FROM PATIENTS OF SEVERE INVASIVE INFECTIONS

The M protein, which is encoded by the *emm* gene, is an important virulence factor for *S. pyogenes*[36]. It has been reported that strains of certain M serotypes are epidemiologically associated with particular clinical syndromes. More than 90 of M protein serotypes have been identified and a molecular approach to identification of *emm* (M protein) genes has also been documented[37]. For example, *emm1*, *emm2* and *emm3* genes encode the M1, M2 and M3 proteins, respectively. The *emm* genotyping is also one of useful tools for the epidemiological investigation. The strains of *emm1* and *emm3* genotype were dominant in causing STSS in almost all of the country[38-41]. However, in tropical northern Australia, the strains of *emm1* genotype were not necessarily dominant[42].

In Japan, *emm49* genotype *S. pyogenes* strains have been isolated from patients of severe invasive GAS infections since 2000[43]. *S. pyogenes* strains of other various *emm* genotypes appeared in Japan and Europe[38,44,45], for example, in Japan, *emm58*, *emm75*, *emm81* and *emm89*[38]. The frequency of *emm3* genotype strains from STSS patients increased rapidly during 1993-4 in Japan and *emm3* strains predominated in STSS patients[38,46]. However, after 1996, the incidence of *emm3* strains isolated from STSS patients suddenly decreased due to unknown reasons.

ANTIMICROBIAL SUSCEPTIBILITY OF ISOLATES FROM PATIENTS OF SEVERE INVASIVE *S. PYOGENES* INFECTIONS

One approach to treat severe invasive *S. pyogenes* infections has been to utilize a combination of penicillin and clindamycin. The rationale is based on the facts that all of the *S. pyogenes* strains isolated are sensitive to penicillin and clindamycin is demonstrated to have a greater efficacy in experimental models of necrotizing fasciitis[47], due to many factors[48-52]. We found that all isolates from patients of severe invasive group A streptococcal infections in Japan were sensitive to ampicillin in addition to cefotaxime, which have a killing activity for the growing bacteria. On the other hand, 1.4% of the isolates were resistant to clindamycin[53]. Clindamycin is more effective for the bacteria at the non-vegetative stage than ampicillin. Strains with *ermB* gene have appeared, which give resistance to clindamycin as well as to telithromycin and erythromycin in the severe invasive infections isolates (Table 1)[53]. These give us important information for the treatment of severe invasive group A streptococcal infections; examination of *ermB* in the isolates is critical for the treatment. Relatively high rates of MLS antibiotic (macrolide, lincosamide, streptogramin B) resistance in France and Italy and very frequent tetracycline resistance were found in almost all Europe countries[44].

SEVERE INVASIVE INFECTIONS BY *STREPTOCOCCUS DYSGALACTIAE* SUBSP. EQUISIMILLIS

Groups G *Streptococcus* (GGS) has shown a similar pathogenic pattern to *S. pyogenes*[54]. It was reported that GGS identified as *S. dysgalactiae* subsp. *equisimilis* also caused streptococcal toxic shock-like syndrome[55-57]; 81.3% (13/16) of the patients in Japan had at least one or more underlying diseases; hypertension (6/16, 33.3%), cancer (5/16, 31.3%), cardiac disease (3/16, 18.8%), cirrhosis (3/16, 18.8%) and one patient each with diabetes mellitus, renal disease, tinea pedis and osteoarthritis[57]. GGS encodes the cell surface M-like protein and at least 23 different forms of the *emm*-like genes (*stg*) have been identified in *S. dysgalactiae* subsp. *equisimilis* and a molecular approach to identification of *stg* genes has been documented (http://www.cdc.gov/ncidod/biotech/strep/emmtypes.htm). In Japan, *S. dysgalactiae* subsp. *equisimilis* carrying a particular *emm* genotype do not always cause invasive diseases[57].

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