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Streptococcus pyogenes isolated in a Tunisian pediatric population: *Emm* types, T types, virulence factors and genes of resistance to macrolide and tetracycline

Sonia Ksia[1], Hanen Smaoui[1], Amel Kechrid[1] and Anne Bouvet[2]

[1]Laboratory of Microbiology, Children's Hospital, Faculty of Medicine of Tunis, Tunisia.
[2]Université Paris Descartes, Laboratoire associé au Centre National de Référence des Streptocoques pour les streptocoques du groupe A, Service de Microbiologie – Hygiène, Hôtel Dieu Assistance Publique – Hôpitaux de Paris, France.
Email: hanen.smaoui@rns.tn

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ABSTRACT

Aims: The aims of our study were to determine epidemiological markers of *S. pyogenes* isolates as well as the antimicrobial activities against these strains and we determined the macrolide and tetracycline resistance genes.
Methodology and Results: We studied the epidemiological markers of 148 *Streptococcus pyogenes* isolates collected from children in Tunisia between October 2000 and December 2006. Antimicrobial susceptibility was performed according to the CA-SFM guidelines. T-serotypes were determined by slide agglutination. Virulence factor genes (pyrogenic exotoxin gene and superantigen gene) and macrolide and tetracycline resistance genes were revealed by PCR method. The *emm* types were determined by sequencing the variable 5' end of the *emm* gene. The predominant markers were T3/12/13/B3264, *emm*22 and *speB*. All isolates were susceptible to β-lactam antibiotics (penicillin and amoxicillin). Resistance to tetracycline was observed in 65 isolates (43.9%), and strains harboured the *tet*(M) gene alone in 95.4% (62/65) or both *tet*(M) and *tet*(L) genes in 4.6% (3/65). Six strains (4%) were resistant to erythromycin among these; five were also resistant to clindamycin. Five strains of genotypes *emm*11, *emm22, emm*28, and *emm76* expressed the constitutive MLS_B phenotype with the presence of the *erm*B gene alone (3 strains) or in association with the *mef*A gene (2 strains). One *emm4* strain expressed the M phenotype and harboured the *mef*A gene alone.
Conclusion, Significance and Impact of Study: This work provided the molecular characteristics of paediatric *S. pyogenes* isolates in Tunisia. Our study affirmed that this micro-organism still susceptible to β-lactam antibiotics but showed an increase in macrolide resistance. We concluded that the epidemiology and the molecular characteristics of *S. pyogenes* strains were different around the world.

Keywords: Streptococcus pyogenes, antimicrobial susceptibility, molecular typing, resistance genes, children

INTRODUCTION

Streptococcus pyogenes or group A streptococci (GAS) is the most common bacterial cause of pharyngitis in children and adults. *S. pyogenes* is also responsible for severe invasive diseases and non-suppurative squeal, especially acute rheumatic fever (ARF) (Cunningham, 2000). It is well recognised that some M serotypes (1, 3, 5, 6 and 18) of *S. pyogenes* are frequently associated with ARF (Kechrid *et al*., 1997).

S. pyogenes produces a number of extracellular proteins including the Streptococcal Pyrogenic Exotoxin (SPE), formerly known as erythrogenic toxin including type A, B and C and streptococcal superantigen (*ssa*) (Murakami *et al*., 2002). The presence of SPE is believed to be associated with pathogenicity and erythematous skin reactions in addition to various immunological and cytotoxic effects (Tyler *et al*., 1992). The type A and C toxins are encoded by the bacteriophage *speA* and *speC* genes respectively. The type B toxin is encoded by the chromosomic *speB* gene (Tyler *et al*., 1992). *Ssa* is a protein that can generate symptoms similar to toxic shock syndrome (TSS) (Murakami *et al.*, 2002). The *emm* gene encoding the M protein has highly heterogeneous 5' ends, and *emm* sequence typing is considered to be the method of choice for GAS strains epidemiological studies (Tyler *et al.*, 1992). This genotyping has facilitated epidemiological investigations on *S. pyogenes* in combination with T-protein antigen typing (Beall *et al.*, 1997).

Until today, *S. pyogenes* remains universally sensitive to penicillin, despite the fact that penicillin has been the drug of choice for streptococcal infections for more than 50 years (Takeaki *et al*., 2008). Resistance to other antimicrobial agents were reported worldwide (Malhotra-Kumar *et al.*, 2005). The prevalence of erythromycin resistant *S. pyogenes* strain had been

*Corresponding author

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reported from an increasing number of countries in recent years (Alos *et al*., 2003). Inducible resistant and constitutive resistant phenotypes have the MLS_B type encoded by the *erm* genes (*ermA* or *ermB*). Clindamycin-susceptible strains have the M phenotype encoded by the *mefA* gene, which codes for a macrolide efflux mechanism (Palavecino *et al*., 2001). Tetracycline resistance is commonly due to ribosomal protection proteins encoded mainly by the *tet*(M) or *tet*(O) genes. The efflux pumps encoded by the *tet*(K) and *tet*(L) genes are uncommon in streptococci (Malhotra-Kumar *et al*., 2005).

In our study, we determined epidemiological markers of 148 *S. pyogenes* strain isolated from Tunisian children including T-serotypes, *emm* sequence types and virulence factor genes (pyrogenic exotoxin gene and superantigen gene). Besides, we tested the antimicrobial activities against these strains and determined the macrolide and tetracycline resistance genes.

MATERIALS AND METHODS

Bacterial strains

From October 2000 to December 2006, a total of 148 non-duplicated isolates of *S. pyogenes* were collected from paediatric patients, aged from one month to sixteen years, in the Laboratory of Microbiology at the Children's hospital of Tunis. Most of the isolated strains (85%) were responsible for non-invasive infections and were obtained from 63 throat samples (43%), 59 pus samples (40%), or other sources (2 nasal and 1 tracheal sample), and 15% were responsible for invasive infections and were obtained from blood-culture (2 samples), articular punctures (15 samples) and other punctures (6 samples).

Identification

Identification of *S. pyogenes* was performed by colony morphology, ß-haemolysis on blood agar (bioMérieux), bacitracin susceptibility (BioRad), detection of pyrrolidonyl arylamidase by rapid test (bioMérieux), and slide agglutination of latex beads coated with specific antibodies (bioMérieux). The strains were stored in brain hearth with 10% of glycerol at –80 °C.

Antimicrobial susceptibility testing

Disc diffusion assays

Isolates were tested on Mueller-Hinton agar (Bio-Rad, France) supplemented with 5% defibrinated horse blood according to the Comité de l'Antibiogramme de la Société Francaise de Microbiologie (CA-SFM) guidelines (Soussy, 2008). Antibiotics tested were penicillin G, amoxicillin, tetracycline, erythromycin, clindamycin, streptomycin, kanamycin, gentamicin, rifampicin, teicoplanin, vancomycin, levofloxacin and bacitracin (Bio-Rad, France). The resistance phenotypes of erythromycin resistant *S. pyogenes* isolates were determined by the double disk test with erythromycin and clindamycin disks as described previously (Kataja *et al*., 1999).

Minimum Inhibitory Concentration determination (MIC)

The minimum inhibitory concentrations (MIC) of penicillin G, amoxicillin, tetracycline, erythromycin, clindamycin and rifampicin were determined for all isolates using the E-test method (AB-BIODISK) on Mueller-Hinton agar supplemented with 5% of defibrinated horse blood agar according to the manufacture instructions.

Characterization of the resistance mechanisms

Detection of tetracycline resistance genes: PCR with specific primer pairs was performed to detect tetracycline resistance genes *tet*(K), *tet*(L), *tet*(M) and *tet*(O) as described previously (Malhotra-Kumar *et al*., 2005).

Detection of erythromycin resistance genes: PCR was performed with buffer, primer sets and electrophoresis conditions as previously described (Morosini *et al.*, 2003). Three references strains of *S. pneumoniae* (P6, P8, P9) and one reference strain of *Staphylococcus aureus* (S7) were used as positive controls for the *ermB*, *mefA*, *ermB* plus *mefA* and *ermA* genes respectively (Ksia *et al*., 2010).

T serotypes

T serotypes were determined by slide agglutination of trypsin digested suspensions of washed bacterial cells in the presence of type-specific antisera as described previously (Sevapharma, Prague, Czech Republic) (Johnson *et al.*, 1996).

emm sequence typing

The *emm* types were determined by sequencing the variable 5' end of the *emm* gene after amplification by PCR with the MF and MR primers as previously described (Loubinoux *et al*., 2004). Sequences were analysed according to the Centers for Disease Control and Prevention (CDC) database: (http://www.cdc.gov/ncidod/biotech /strep/doc. htm).

Detection of *speA*, *speB*, *speC* and *ssa*

Multiplex PCR was performed to detect the presence of the s*peA*, *speB*, s*peC* and *ssa* genes using specific primers as previously described (Chatellier *et al*., 2000).

RESULTS

Susceptibility testing revealed that all tested *S. pyogenes* isolates were susceptible to penicillin G and amoxicillin. MICs of penicillin G and amoxicillin ranged from 0.004 to 0.023 mg/L and 0.016 to 0.094 mg/L respectively (Table 1). All *S. pyogenes* isolates were susceptible to rifampicin, teicoplanin, vancomycin and levofloxacin. For aminoglycoside antibiotics, a high level of resistance to

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kanamycin and streptomycin was observed in 4 and 2 isolates, respectively. We found a high rate of tetracycline resistance in 65/148 (43.9%), MIC of tetracycline ranged from 0.064 to 32 mg/L. Of the 65 tetracycline resistant isolates, 95.4% (62/65) carried the *tet*(M) gene and 4.6% (3/65) carried both *tet*(M) and *tet*(L) genes (Figure 1).

Table 1: Minimum inhibitory concentrations of 148 isolates of *S. pyogenes*.

Antimicrobial agent	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Range (mg/L)
Penicillin G	0.008	0.016	0.004 – 0.023
Amoxicillin	0.023	0.032	0.016 – 0.094
Rifampicin	0.023	0.047	<0.016 – 0.125
Erythromycin	0.064	0.125	<0.016 - >256
Clindamycin	0.094	0.19	<0.016 - >256
Tetracycline	0.064	24	0.064 – 32

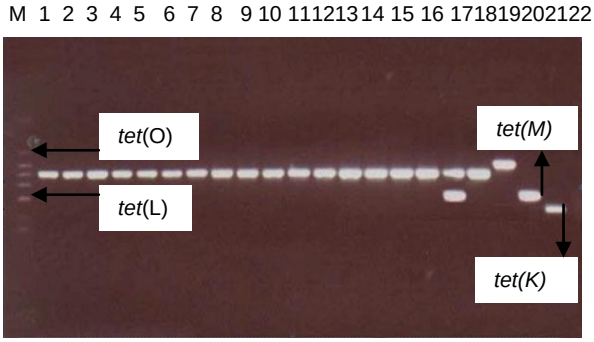

Figure 1: PCR analysis of the 65 *S. pyogenes* tetracycline-resistant isolates. Legend: M: DNA molecular weight marker; Lane 1 to 16: tetracycline resistant strains harbouring *tet*(M); Lane 17: tetracycline resistant strain harbouring both *tet*(M) and *tet*(L); Lane 18: control strain for detection of *tet*(M); Lane 19: control strain for detection of *tet*(O); Lane 20: control strain for detection of *tet*(L); Lane 21: control strain for detection of *tet*(K); Lane 22: negative marker.

Of all isolates tested, only 5 (3.4%) were resistant to both erythromycin and clindamycin, and were assigned to the $cMLS_B$ phenotype and harbored the *erm*B gene alone (3 strains) or in association with the *mef*A gene (2 strains). Only one isolate (0.7%) was resistant to erythromycin alone and assigned to the M phenotype and harbored the *mef*A gene. The strains with $cMLS_B$ phenotype had higher MICs for erythromycin and clindamycin than those with M phenotype. In fact, for all the $cMLS_B$ isolates, the MICs of erythromycin and clindamycin were >256 mg/L for these two antibiotics. For the M one, the MICs of erythromycin and clindamycin were 12 mg/L and 0.19 mg/L respectively. In our study, a linkage between *tet*(M) and *ermB* genes was found in one isolate. One strain was resistant to bacitracin (0.7%), and erythromycin, clindamycin and had high levels of resistance to streptomycin and kanamycin.

For T serotyping, 47 strains (31.7%) were non-typables and in total, 20 different T serotypes were identified, of which T3/12/13/B3264 (18.2%), T8/25/Imp19 (10.8%), T1 (8.1%) and T9 (5.4%) were predominant. Among these, T1 and T9 types were predominant in invasive strains, while T3/12/13/B3264 and T8/25/Imp19 prevailed in non-invasive strains. For *emm* typing, 35 different types were identified, of which *emm22* (19.6%), *emm76* (12.8%), *emm1* (8.1%) *emm9* (6.7%) and *emm118* (6.7%) were predominant. The most frequent types among invasive strains were *emm76, emm9* and *emm1,* whereas *emm76* and *emm22* were predominant among non-invasive strains. The T agglutination patterns associated with each of the *emm* types were identical to those of other countries, except for a few combinations (Table 2). Five different *emm* types and 4 different T types were found among the six erythromycin resistant isolates. The bacitracin resistant strain was of *emm28* and T28 types.

The results of multiplex PCR to detect pyrogenic exotoxin genes and superantigens (Figure 2) indicated that the *speB* gene was detected in all strains of *S. pyogenes* tested and was alone in 36.5% of cases. The frequency of detection of each toxin gene among all strains tested was 36.5% for *ssa*, 22.3% for *speC* and 17.5% for *speA*. In our study, *ssa* gene was the most frequent among invasive strains (30.4%) and both *ssa* and s*peC* genes among non-invasive (37.6 % and 23.2 % respectively).

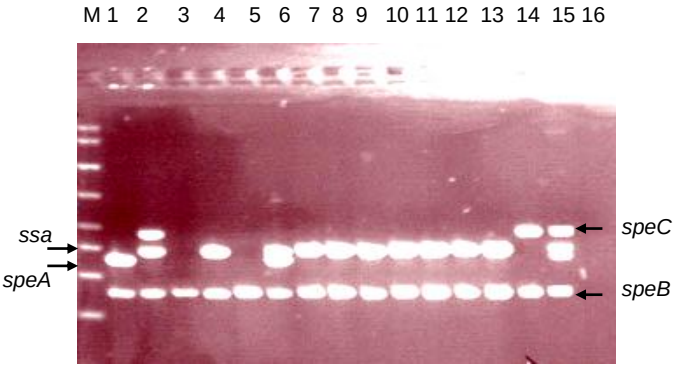

Figure 2: Detection of exotoxin pyrogenic genes and streptococcal superantigen by PCR multiplex. Legend M: DNA molecular weight marker; Lane 1 to 14: strains tested harbouring different toxin genes; Lane 15: control strain for detection of 4 toxin genes; Lane 16: negative marker.

The analysis of the toxin genes in all strains revealed 7 different toxin-gene profiles (Table 2) and the most frequent association was *ssa*/*speB*. We primarily focused on the 3 most commonly encountered *emm* types 22, 76 and 1. In fact, all *emm1* strains harboured *speA* gene, while *emm76* and *emm22* harboured *ssa* gene frequently (24% and 46.3% respectively).

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Table 2: Correlation between *emm* and T-types of *S. pyogenes* strains and toxin gen.

emm types (N)*	N* of isolates	T types (N)*	*speA*	*speB*	*speC*	*ssa*
1 (12)	9	T1 (8), NT(1)	+	+	-	-
	2	T1(2)	+	+	+	-
	1	T1(1)	+	+	-	+
3 (2)	2	NT(2)	+	+	-	+
4 (1)	1	NT(1)	-	+	-	+
9 (10)	7	T9(6), 14(1)	-	+	+	-
	3	T9(3)	-	+	-	-
11 (4)	2	T11(1),T8/25/Imp19(1)	-	+	+	-
	2	T11(1),T8/25/Imp19(1)	+	+	-	-
12 (5)	5	T12(4), T12/13(1)	-	+	-	-
18 (1)	1	T9(1)	-	+	+	-
22 (29)	3	T3/12/13/B3264(2), NT(1)	+	+	-	-
	19	T3/12/13/B3264(17), T12(2)	-	+	-	+
	5	T3/12/13/B3264(5)	-	+	+	+
	2	T3/13/B3264(2)	-	+	-	+
28 (3)	2	T3/13/B3264(2)	-	+	-	-
	1	T28(1)	-	+	+	-
33 (5)	5	NT (4), T8/25/Imp19(1)	-	+	+	+
41	1	T6(1)	-	+	+	+
42 (2)	2	T8/25/Imp19(2)	-	+	-	-
44/61 (2)	2	NT(1), T12/27/44(1)	-	+	-	-
49 (2)	2	T8/25/Imp19(1), NT(1)	-	+	-	-
60 (5)	4	T8/25/Imp19(1), T2/8/25/Imp19 (1) T4(2)	-	+	-	-
	1	T4(1)	-	+	+	-
66 (1)	1	T4(1)	+	+	-	-
73 (1)	1	NT(1)	-	+	+	-
74 (1)	1	NT(1)	-	+	+	-
75 (8)	8	T8/25/Imp19(6), T3/13/B3264(1), NT(1)	-	+	-	-
76 (19)	14	NT(13), T12/44(1)	-	+	-	+
	3	T1(1), NT(2)	+	+	-	-
	2	NT(2)	-	+	-	-
77 (3)	3	T2(1), T13(1), T8/25/Imp19(1)	-	+	-	-
78 (2)	1	NT(1)	-	+	-	+
	1	NT(1)	-	+	+	-
83 (1)	1	NT(1)	-	+	-	-
85 (1)	1	T3/12/13/B3264(1)	-	+	-	-

*N: number of isolates

(continued)

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emm types (N)*	N* of isolates	T types (N)*	*speA*	*speB*	*speC*	*ssa*
87 (1)	1	NT(1)	+	+	-	+
92 (1)	1	T11(1)	-	+	-	-
98 (2)	2	NT(1), T8/25/Imp19(1)	-	+	+	-
106 (7)	6	T3(1), NT(1), T3/13/B3264(4)	-	+	-	-
	1	T11(1)	-	+	+	-
108 (1)	1	NT(1)	-	+	-	-
118 (10)	10	NT(7), T3(1), T5/27/44(2)	-	+	-	-
112 (1)	1	T8/25/Imp19(1)	-	+	-	-
115 (1)	1	NT(1)	+	+	+	-
116 (1)	1	T8/25/Imp19(1)	-	+	-	+
122 (1)	1	NT(1)	+	+	+	-
stD432 (1)	1	T8/25/Imp19(1)	-	+	-	+

*N: number of isolates

DISCUSSION

Susceptibility testing showed that all *S. pyogenes* isolates tested were susceptible to all ß-lactams. Resistance to penicillin G had not been reported in *S. pyogenes* in any country worldwide (Takeaki *et al*., 2008). The rate of resistance to tetracycline among our isolates were 43.9% (65/148).This rate was 93% in Tunisia between 1988 and 1990 (El Bour *et al*., 1993). Resistance to tetracycline had been reported in some countries: 25.4% in Spain (Rivera *et al*., 2006), 50% in Brazil (De Melo *et al*., 2003) and 80% in Korea (Sook *et al*., 2007). In many countries, tetracycline was widely used as treatment for a variety of human and veterinary infections implying high total level of consumption and contributing to the emergence of this resistance among *S. pyogenes* isolates around the world (De Melo *et al*., 2003). Much works has been done to characterize the mechanism of tetracycline resistance in *S. pyogenes* in which only *tet*(M) gene had been commonly identified (Giovanetti *et al*., 2003). In our collection, all the tetracycline resistant isolates carried the *tet*(M) gene, alone in 95.4% or associated with *tet*(L) in 4.6% of strains. *tet*(O) and *tet*(K) genes were absent. The linkage between *tet*(M) and *tet*(L) genes had not been reported previously. In Spain, *tet*(M) was present in 56.2%, *tet*(O) in 18.7% and one isolate carried both these genes (Rivera *et al*., 2006). In Denmark, *tet*(M) was detected in 23 of 31 tetracycline resistant isolates (Hammerum *et al*., 2003) and in study of Luca-Harari and co-workers (2008), *tet*(M) was detected in 16 of 23 tetracycline resistant isolates and *tet*(O) was detected in 7 of them (Luca-Harari *et al*., 2008). Five among the six erythromycin resistant isolates were also resistant to clindamycin and assigned to the $cMLS_B$ phenotype. This phenotype was the most common among Tunisian *S. pyogenes* isolates (Hraoui *et al*., 2010). However, in Germany and Chile, the most common phenotype of resistance to macrolides was the M phenotype (Palavecino *et al*., 2001; Sauermann *et al*., 2003). An important increase in the prevalence of erythromycin resistance in *S. pyogenes* had been reported in some countries (Alos *et al*., 2003). The rates of resistance to erythromycin were 21.5% in Korea (Sook *et al*., 2007) and 24% in Germany (Grivea *et al*., 2006). The majority of erythromycin and clindamycin resistant strains were multiresistant to other antibiotics such as tetracycline, streptomycin and kanamycin (Mihaila-Amrouche *et al*., 2004). The M phenotype strain when assayed by PCR showed the presence of the *mefA* gene responsible for the efflux system. Three of the five $cMLS_B$ phenotype isolates harboured the *ermB* gene and 2 harboured *ermB* plus *mefA* genes. In Germany, the majority of *S. pyogenes* strains with $cMLS_B$ phenotype harboured *erm*A gene alone or associated with *mef*A gene (Grivea *et al*., 2006).

A linkage between *ermB* and *tet*(M) was well known in *Streptococci* in many studies, mainly due to the widespread occurrence in these organisms of genetic elements resulting from the insertion of *ermB* containing DNA into a conjugative transposons of the Tn916 family which typically carryed *tet*(M) (Brenciani *et al*., 2007). Tetracycline resistance determinants were genetically linked to *erm* genes rather than to *mefA* (Brenciani *et al*., 2007). These linkage between *tet*(M) and *ermB* gene was also found in Spain (Rivera *et al*., 2006) and in our study in one isolate. In many studies, tetracycline resistance

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was considered an important co-factor in the selection of erythromycin resistance. In contrast, in other studies a linkage between *tet*(O) with either *ermA* or *mefA* genes was found eg. in Italy (Giovanetti *et al*., 2003, Bacciaglia *et al*., 2007), in Spain (Rivera *et al*., 2006) and in Denmark (Hammerum *et al*., 2003). All our *S. pyogenes* isolates were susceptible to levofloxacin, rifampicin, teicoplanin and vancomycin as reported (Sauermann *et al*., 2003). One strain (0.7%) resistant to bacitracin was also resistant to streptomycin, kanamycin, clindamycin and erythromycin, as previously reported from Belgium and France (Malhotra-Kumar *et al*., 2003; Mihaila-Amrouche *et al*., 2004). It had been demonstrated that the majority of bacitracin-resistant *S. pyogenes* strain are clonal and the resistance to this antibiotic was believed to result from an overproduction of undecaprenol kinase encoded by the *bacA* gene (Malhotra-Kumar *et al*., 2003).

In our study, 20 different T types were observed among the 148 GAS isolates tested and the most prevalent ones were T3/12/13/B3264, T8/25/Imp19, T1 and T9, and, 31.7% of the isolates were non-typeable. In Sweden, T3/13/B3264 type was the most prevalent (Darenberg *et al*., 2007). In our study, T1 and T9 types were the dominant types among invasive strains and T3/12/13/B3264 and T8/25/Imp19 among non-invasive. A previous study characterising isolates from Tunisia showed that the most frequent T types among invasive strains were T3/13/B3264 and T1, for non-invasive, T3/13/B3264 and T4 were predominant (Hraoui *et al*., 2010). In Japan, the dominant types were T12, T1 and T4 for the isolates from throats and T28, TB3264 and T non-typeable for the isolates from other sources (Tanaka *et al*., 2002).

In the present study, 35 different *emm* types were identified and the most prevalent ones were *emm22*, *emm76*, *emm1*, *emm9* and *emm118*. Worldwide, *emm* typing showed divergent results: the predominant ones were *emm118*, *emm42* and *emm1* in an adult population in Tunisia (Hraoui *et al*., 2010); *emm1* in Germany (Wahl *et al*., 2007); *emm1*, *emm3* and *emm4* in France (Bidet *et al*., 2007); *emm4*, *emm12* and *emm1* in Taiwan (Chiou *et al*., 2004); *emm1*, *emm28* and *emm89* in Denmark (Ekelund *et al*., 2005). In our study, *emm76, emm9* and *emm1* were the most frequent types among invasive strains and *emm76* and *emm22* types among non-invasive. In a previous study in Tunisia (Hraoui *et al*., 2010), *emm1, emm76* and *emm118* were the most frequent types among invasive strains. In Denmark, *emm1* was the most frequent type among invasive strains, whereas *emm* 4, 6 and 12 among non-invasive ones (Ekelund *et al*., 2005). In Sweden, the most frequent *emm* types among invasive infections were *emm*89, 81 and 28 (Darenberg *et al*., 2007). In contrast with these results, a study of Sagar and co-workers found that there was no significant association between *emm* types and strains origin (Sagar *et al*., 2008).

The T agglutination patterns associated with each of the *emm* types found were identical with those reported by Johnson and co-workers (2006) except for few combinations. These results reflect a wide geographic distribution of a specific strain (Beall *et al*., 1997). Most of studies found that each phenotype or genotype of resistance to macrolides was related to a specific *emm* type. In our study, the M phenotype strain was of *emm4* type. This was also found in Spain and Germany (Alberti *et al*., 2003; Grivea *et al*., 2006). The correlation between *emm* types and genotypes of macrolide resistance differs around the world. Our study shows that $cMLS_B$ phenotype isolates were of different *emm* types. In Spain and in Italy (Alberti *et al*., 2003; Zampaloni *et al*., 2003), *emm22* was predominant in $cMLS_B$ strains. In Japan (Takeaki *et al*., 2008), 21.3% of strains with *mefA* gene were of *emm1* type and 11.7% of strains with *ermB* gene were of *emm12* type. Resistance to tetracycline among the *emm76* type isolates in the present study was high (18/19), while, none of the *emm1* isolates was resistant to tetracycline. Tewodros and co-workers (2005) found that *emm1* isolates were less likely resistant to tetracycline. In another study, *emm*1 and *emm29* isolates were associated with resistance to tetracycline (Barrozo *et al*., 2003).

Detection of pyrogenic exotoxin genes and streptococcal superantigen genes indicated that the *speB* gene was found in all strains of *S. pyogenes* tested. The same results were reported in other studies (Tyler *et al*., 1992; Hraoui *et al*., 2010). The other most frequent toxin gene was *ssa,* it accounted for 36.5% of all strains and for 30.4% of invasive ones. Similar results were found in China and Japan (Murakami *et al*., 2002; Chang *et al*., 2011). Among non-invasive strains, both *ssa* and *speC* were the most frequent genes (37.6% and 23.2% respectively). In previous studies, *SpeA* was the most prevalent gene among invasive strains (Murakami *et al*., 2002; Hraoui *et al*., 2010).

A variety of studies reveal that the distribution of *emm* genotypes was related to pyrogenic exotoxin genes and streptococcal superantigen (Murakami *et al*., 2002). In our study, s*peA* gene was the most common among all *emm1* isolates, *ssa* gene in the majority of *emm22* and *emm76* isolates and *speC* gene among *emm9* strains. In Germany, *speA* and *speC* were found in *emm1* and *emm4* respectively (Wahl *et al*., 2007), in Japan, s*peA* gene was most frequent among *emm1* and *emm3* isolates and *ssa* gene among *emm3* and *emm4* isolates (Murakami *et al*., 2002). In Denmark and Norway, the *speA* gene was most frequent among *emm1* isolates (Luca-Harari *et al*., 2008). Almost every *emm* type was characterized by the predominance of 1 or 2 toxin-gene profiles, clearly supporting the concept of clonal distribution (Schmitz *et al*., 2003). In our study, toxin-gene profiling of *emm76* and *emm22* isolates revealed that the majority of the strains carried both *speB* and *ssa* simultaneously.

The bacitracin resistant strain was isolated from pus sample, it is of serotype T28, *emm28* type, $cMLS_B$ phenotype and multiresistant to antibiotics such as strains find in France. This multiresistant clone of *emm* type 28 is frequently associated with invasive infections and is reported as the second-most-common invasive type (Mihaila-Amrouche *et al*., 2004).

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CONCLUSION

Susceptibility testing revealed that all tested *S. pyogenes* isolates were susceptible to penicillin G, have high rate of tetracycline resistance with predominance of *tet*(M) gene, low rate of erythromycin resistance with predominance of *erm*B gene. For T serotyping, *emm* typing and pyrogenic exotoxin genes and superantigens T3/12/13/B3264, *emm22* and *speB* and *ssa* were predominant.

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