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ORIGINAL ARTICLE

Molecular *emm* typing of Bulgarian macrolide-resistant *Streptococcus pyogenes* isolates

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ABSTRACT

Group A streptococcus (GAS) is a human pathogen causing a broad range of infections, linked with global morbidity and mortality. Macrolide resistance rates vary significantly in different parts of the world. Driving factors of the emergence and spread of resistant clones are not clearly understood. We investigated 102 macrolide-resistant GAS strains collected during the period 2014–2018 from various clinical specimens from Bulgarian patients. Strains were characterized by the presence of *mefA/mefE, ermA*, and *ermB* using polymerase chain reaction and sequencing for *mefA/mefE*. Resistant strains were studied by *emm* sequence typing and *emm*-cluster system. Most prevalent *emm* types among the macrolide-resistant GAS strains were *emm*28 (22.55%), *emm*12 (17.65%), and *emm*4 (16.66%). Almost all (87.25%) of the macrolide-resistant isolates harboring *ermB* were *emm*28. The isolates that carried *ermA* were predominantly *emm*12 (38.24%) and *emm*77 (38.24%), with fewer *emm*89 (23.53%). The isolates harbored predominantly *mefE (49 isolates)* and only 9 strains carried *mefA*. The most prevalent *emm* clusters among the GAS isolates were E4 (40.20%), A-C4 (17.65%), and E1 (16.66%). The study's results suggest that dissemination of specific clones in GAS population may also be the reason for the increasing macrolide-resistance rate in our country.

KEYWORDS

Streptococcus pyogenes, *emm* types, macrolide resistance

INTRODUCTION

Group A streptococcus (GAS), or *Streptococcus pyogenes*, is a human pathogen responsible for a broad range of infections and is an important cause of global morbidity and mortality [1]. Penicillin has been the drug of first choice for the treatment of infections caused by this microorganism since the 1940s and is still effective [2, 3]. However, in the past few years, 20%–40% failure has been reported for this treatment [4]. Macrolides are important alternatives for treatment of patients who are allergic to penicillin or in cases of penicillin treatment failure [5]. The use of clindamycin for life-threatening invasive infections may reduce the mortality considerably [6]. Unfortunately, the use of these antibiotics is limited due to the increasing macrolide resistance in some countries [7–11]. A new method for understanding the epidemiology of the GAS types is *emm* cluster typing, based on the M protein, which groups *emm* types into 48 different functional *emm* clusters on the basis of their structural properties [12].

This study aimed to determine the *emm* types distribution of Bulgarian clinical GAS isolates harboring macrolide-resistance determinants.

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MATERIAL AND METHODS

A collection of 102 macrolide-resistant GAS strains was included in this study. The isolates originated from throat swabs ($n = 68$) (with a diagnosis of tonsillopharyngitis and scarlet fever), peritonsillar abscess punctures ($n = 5$), ear punctures ($n = 11$), and wound secretion ($n = 18$), during the period 2014–2018. The age range of the patients was 3–85 years. Strain identification and the polymerase chain reaction (PCR) protocols used for detection of the macrolide resistance genes (*ermB*, *ermA*, and *mefA/mefE*) were performed as previously described [8]. The amplified segments with primers for *mefA/E* were sequenced in both strands and analyzed with DNAMAN version 8.0 Software (Lynnon BioSoft, Vaudreuil-Dorion, Canada).

The genotypes were determined by sequence analysis of the variable 5″ region of the *emm* gene after amplification by PCR in accordance with the protocol of the Centers for Disease Control and Prevention (https://www.cdc.gov/streplab/groupa-strep/emm-typing-protocol.html), using the following primers F: GGGAATTCTATTSGCTTAGAA AATTAA, R: GCAAGTTCTTCAGCTTGTTT [13]. The amplification products were separated in a 2% agarose gel for 90 min at 120 V, stained with ethidium bromide (0.5 mg/L), and detected by UV transillumination (wavelength = 312 nm). The gene *emm* was sequenced using a BigDye Terminator v3.1 Cycle Sequencing Kit and BigDye Terminator v1.1 and v3.1 5X Sequencing Buffer in an Applied Biosystems 3130xl Genetic Analyzer. The data were analyzed and edited using Chromas version 2.1. The 5' ends of the *emm* sequences were compared to sequences in the CDC database (http//:www.cdc.gov/ncidod/biotech/strep/strepblast.html) and *emm* genotypes were clustered according to the data in the CDC database.

RESULTS

The distribution of the *emm* types in 102 GAS isolates with presence of different macrolide resistance genes is presented in Table I. As shown, the most prevalent *emm* types among all the tested strains were *emm*28 (22.55%), *emm*12 (17.65%), and *emm*4 (16.66%), accounting for over 50% of isolates. About 87.25% of the macrolide-resistant isolates harboring *ermB* were *emm*28. The isolates that carried the *ermA* gene were predominantly *emm*12 (38.24%) and *emm*77 (38.24%), and fewer were *emm*89 (23.53%). The isolates harboring *mefA/mefE* were the most heterogeneous according to the *emm* types (Table I). Sequencing the *mef* genes of our isolates revealed that 49 strains were positive for *mefE*, 100% identical with the corresponding segment of published sequence of *mefE* gene with accession number EU870854.1, and 9 strains showed presence of *mefA* genes (100% identical with published sequence accession number AF227521.1). Various *emm* types were presented in strains with *mefE* and *mefA*, respectively, where no clonal association was found. All the *emm* types in this study belonged to six *emm* clusters. The most prevalent clusters among the Bulgarian macrolide-resistant GAS isolates were E4 (40.20%), A-C4 (17.65%), and E1 (16.66%), observed in over 50% of the examined isolates.

DISCUSSION

Increasing macrolide resistance is mostly associated with increased local consumption of macrolide antibiotics or with the spread of specific macrolide-resistant clones [14, 15]. According to the ECDC, Bulgaria was the seventh largest

Table I. Distribution of *emm* types, *emm* clusters, and macrolide-resistance genes observed in examined 102 GAS strains

emm type	No. of isolates [*n* (%)]	*emm* Cluster	Genes encoded macrolide resistance in the tested 102 isolates		
			mefA/mefE	*ermA*	*ermB*
			58 (56.86)	19 (18.63)	25 (24.50)
*emm*1	12 (11.76)	A-C3	12 (20.69)	0	0
*emm*2	4 (3.92)	E4	4 (6.9)	0	0
*emm*3	12 (11.76)	A-C5	11 (18.96)	0	1 (4)
*emm*4	17 (16.66)	E1	17 (29.31)	0	0
*emm*6	2 (1.96)	M6	2 (3.45)	0	0
*emm*12	18 (17.65)	A-C4	8 (13.79)	8 (42.10)	2 (8)
*emm*28	23 (22.55)	E4	1 (1.72)	0	22 (88)
*emm*77	8 (7.84)	E4	0	8 (42.10)	0
*emm*89	6 (5.88)	E4	3 (5.17)	3 (15.79)	0
	102 (100)		58 (100)	19 (100)	25 (100)

Note: GAS: group A streptococcus.

consumer of macrolides, lincosamides, and streptogramins in the community and hospital sector in Europe in 2017 [16]. In parallel, the macrolide-resistance rate in Bulgaria has increased from 23% in 2013–2014 to nearly 40% in 2015–2016 [8]. The fluctuations in the macrolide resistance rates do not always depend on the macrolide consumption in a society [17]. It is also possible that increase in the macrolide resistance in our area is associated with the widespread of specific clones, like *emm*28 possessing the *ermB* gene. Over the past 20 years, there have been studies showing similar clonal distribution of the multiresistant *emm*28 clone in other European countries, such as Spain, France, and Belgium [18–20]. The current work showed that macrolide-resistant isolates carrying the *ermA* gene are predominantly presented in genotypes *emm*77 and *emm*12, similar to the results from earlier Serbian studies [21]. A large-scale study showed that *emm*28 and *emm*12 were included in the major seven identified *emm* types in Europe and North America, especially *emm*28 in Denmark, Finland, and Germany [22]. After 2013, *ermB* has been reported in 22.55% of resistant strains [8] and this study showed that almost 90% of GAS-harboring *ermB* belonged to *emm*28. This data indicates that the clonal association is the other reason for the rise in macrolide-resistance levels in Bulgaria.

All the macrolide-resistant *emm* types found in this study have been included in a new 30-valent M protein-based vaccine, which is under development [23]. Recently, the *emm* cluster system can give new understanding and support to vaccine design and evaluation of GAS infections and their complications [12]. In this study, we identified six *emm* clusters among the macrolide-resistant GAS strains. The most prevalent *emm* clusters among the macrolide-resistant isolates from our area, which were E4 (40.20%), A-C4(17.65%), and E1(16.66%), showed similarity with the *emm* cluster identified in pharyngeal and non-pharyngeal pediatric isolates from Greece [24], but not in the Pacific region [25].

CONCLUSIONS

To our knowledge, this is the first study that applies the *emm* cluster typing system in Bulgarian macrolide-resistant GAS isolates. We also suggest that clonal dissemination among the GAS population (like *emm*28 possessing the *ermB* gene) is responsible for the increasing macrolide-resistance rate in our country.

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Conflict of Interest: The authors declare no conflict of interest.

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