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FULL PAPER

Antimicrobial resistance rates of *Streptococcus pyogenes* in a Greek tertiary care hospital: 6-year data and literature review

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SUMMARY

Streptococcus pyogenes is responsible for various clinical manifestations in patients of all ages worldwide. Worryingly, an increase in antibiotic resistance rates of *S. pyogenes* has been observed in many countries. In the present study, 6-year data are presented regarding the antibiotic resistance rates of *S. pyogenes* in our hospital. During this period, a total of 52 *S. pyogenes* isolates were recovered from 52 patients and antimicrobial susceptibility testing was performed for 49 isolates. All were susceptible to penicillin, ampicillin, cefotaxime, ceftriaxone, linezolid, moxifloxacin, rifampicin, vancomycin, teicoplanin, and tigecycline. Erythromycin and clindamycin resistance rates were 20.4% and 18.8% respectively. Resistance rates to tetracycline were 40.8%, to chloramphenicol 6.9%, and to levofloxacin 2%. Since macrolides are recommended as an alternative treatment in case of allergy to β-lactams, the high macrolide resistance rates are causing concern. Because different phenotypic antimicrobial patterns for *S. pyogenes* have been observed in different geographic areas, epidemiological data is of considerable value for the appropriate treatment choices.

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INTRODUCTION

Streptococcus pyogenes is a Gram-positive, facultative anaerobic, β-hemolytic, Lancefield group A streptococcus (GAS) (Kebede *et al.*, 2021). *S. pyogenes* harbors the *emm*-gene that encodes for the M-protein which is attached to the bacterial cell wall (Castro & Dorfmueller, 2021), binds to host immune cells and inhibits phagocytosis, contributing to GAS protection and survival (Andrina *et al.*, 2020; Villalón *et al.*, 2021). Moreover, the M-protein shows cross-reactivity to human organs and especially heart tissue by inducing immunoglobulin production against myosin (Castro & Dorfmueller, 2021). More than 200 *emm* types of GAS have been described, encoding more than 120 designated M-proteins. Thus, the development of an effective and safe vaccine with the M-protein as antigenic target still remains a real challenge (Castro & Dorfmueller, 2021).

Key words:
Streptococcus pyogenes, β-lactams, macrolides.

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Humans are the only natural reservoir of *S. pyogenes*. People of any age can be affected, even though young children and the elderly are at higher risk; males are more frequently affected than females (Avire *et al.*, 2021). A wide spectrum of clinical manifestations can be due to GAS infections. These include necrotizing fasciitis (NF), sepsis, septic arthritis, streptococcal toxic shock syndrome (STSS), pharyngotonsillitis, erysipelas, impetigo, cellulitis, and scarlet fever (Avire *et al.*, 2021; di Pietro *et al.*, 2021). Moreover, a GAS infection may lead to late autoimmune reactions such as acute rheumatic fever (ARF), rheumatic heart disease (RHD), and acute glomerulonephritis (AGN) (Avire *et al.*, 2021). Transmission of GAS most often occurs by respiratory droplets and direct contact with skin sores, even though food-borne transmission has also been described (Avire *et al.*, 2021).
During the 20th century, the incidence of GAS infections declined in some regions but remains a global public health problem, especially in developing countries (Avire *et al.*, 2021). A study by WHO reported that more than 1.78 million people are infected annually, with a mortality rate that approaches 517,000 deaths per year (WHO, 2005). More recently there have also been reports from the European region to the WHO of an increase in cases of invasive group A streptococ-

cus (iGAS) disease and scarlet fever. An increase in iGAS-related deaths has also been reported in some European countries, with children less than 10 years of age representing the most affected age group (https://www.ecdc.europa.eu/en/news-events/increase-invasive-group-streptococcal-infections-among-children-europe-including). Therefore, the prevention and targeted treatment of GAS infections are considered of even higher importance.

To date, antibiotics (mainly β-lactams) are the only realistic and effective treatment approach. Penicillin and amoxicillin are the first choices (Kebede *et al*., 2021) whereas cephalosporins are recommended as alternative options (Khademi *et al*., 2020). Unfortunately, an increase in antibiotic resistance rates has recently been reported from many parts of the world, with variable phenotypic patterns reported from different countries (Khademi *et al*., 2020; Babiker *et al*., 2021; Ikebe *et al*., 2021).

In the present study we present six-year data regarding the antibiotic resistance rates of *S. pyogenes* in a tertiary-care teaching hospital in Thessaloniki in Northern Greece. Furthermore, a literature review was conducted in an attempt to present recent available knowledge regarding the emergence of antibiotic resistance among *S. pyogenes* strains globally.

Literature review on antibiotic resistance of S. pyogenes

According to current treatment guidelines, penicillin and β-lactams are considered the primary treatment option for uncomplicated GAS infections (Andrina *et al*., 2020). In case of severe invasive manifestations, clindamycin should be used as an adjunctive treatment to β-lactam antibiotics (Ikebe *et al*., 2021). For patients allergic to β-lactams, macrolides and lincosamides are the alternative options (Berbel *et al*., 2021). Even though the susceptibility rates to β-lactams (especially to penicillin) remain high, resistance to macrolides and lincosamides has been reported in many countries (Barros, 2021) and associated with increased prescription (Messina *et al*., 2020).

Specific emm genotypes and resistance genes are correlated with antibiotic non-susceptibility in GAS (Ikebe *et al*., 2021; Chehelgerdi & Ranjbar, 2021) and the underlying mechanisms include target site modifications, efflux pump over-expression and drug inactivation (Barros, 2021). The respective genes are summarized in *Table 1*.

Among the GAS antibiotic resistance phenotypes, macrolide resistance is more commonly observed (Tsai *et al*., 2021) and is mainly due to two distinct mechanisms. The first is due to macrolide-specific efflux pumps encoded by the *mef* genes, conferring resistance to macrolides only (M phenotype - resistance to macrolides) (Berbel *et al*., 2021). The second is target modification of the 23srRNA by a methylase encoded by *erm*A(TR) or *erm*B genes (Berbel *et al*., 2021). This contributes to reduced binding of erythromycin and clindamycin and is known as the MLS_B phenotype (resistance to Macrolides, Lincosamides and Streptogramin B). Methylase expression can be either constitutive (cMLSB) or inducible (iMLSB) (Berbel *et al*., 2021). Many studies have been conducted on macrolide-resistance rates among GAS strains and have shown variable results in different countries, including China (98.3%) (Li *et al*., 2020a), Japan (39.8%) (Ikebe *et al*., 2021), Ethiopia (21.4%) (Kebede *et al*., 2021), Greece (15.4%) (Grivea *et al*., 2020), Hungary (10.5%) (Gajdács *et al*., 2020), Spain (8.9%) (Villalón *et al*., 2021), Iran (5.4%) (Khademi *et al*., 2020), and Korea (3.2%) (Kim *et al*., 2019). Regarding the *emm* types, it has been shown that the *emm*1 genotype is susceptible to macrolides in most studies, whereas *emm*28 presented the highest resistance rates (Tsai *et al*., 2021; Grivea *et al*., 2020; Li *et al*., 2020b; Ubukata *et al*., 2020).

Table 1 - *Active antimicrobials against S. pyogenes and mechanisms of resistance.*

Antibiotic category	*Antibiotic mechanism of action*	*Resistance mechanism*	*Responsible gene(s)*
β-*lactams*	Inhibit peptidoglycan synthesis by binding to penicillin-binding proteins (pbp)	Target site modification	*pbp2x*
Macrolides	Inhibit protein synthesis by binding to the 30S ribosomal subunit	Target site modification	*erm*
		Efflux pump	*mef*
Lincosamides	Inhibit protein synthesis by binding to the 50S ribosomal subunit	Drug inactivation	*lnuB*
		Efflux pump	*lsa*
Fluoroquinolones	Inhibit type II topoisomerases (DNA gyrase and topoisomerase IV)	Target site modification	*gyrA* and *parC*
Tetracyclines	Inhibit protein synthesis by binding to the 30S ribosomal subunit	Drug inactivation	
		Efflux pump	*tetA, tetC, tetD, tetE, tetG, tetH.*
		Ribosomal protection	*tetI* *tetM, tetO*
Vancomycin	Inhibits peptidoglycan synthesis by binding to D-alanine-D-alanine portion of the cell wall	Target site modification	*vanG*

Table 2 - *S. pyogenes isolations per year.*

Year	Samples							N	N %
	Pharyngeal	Ear	Pleural fluid	Surgical wound	Wound	Blood	Tissue		
2016	2	2	1	0	0	1	0	6	11.6
2017	7	1	0	2	2	0	0	12	23.0
2018	2	1	0	0	3	1	0	7	13.5
2019	3	2	0	1	4	3	1	14	26.9
2020	0	3	1	1	0	5	0	10	19.2
2021	0	0	0	1	0	2	0	3	5.8

Resistance to lincosamides, including clindamycin, is also commonly observed. The presence of *lnuB* and *lsa* genes has been associated with such phenotypes because of drug inactivation and efflux, respectively (Barros, 2021). Moreover, the *lsa* gene confers resistance to streptogramin A and pleuromutilins (Barros, 2021). Resistance to lincosamides has been reported from China (87.8%) (Li *et al*., 2020b), Ethiopia (50%) (Kebede *et al*., 2021), Iran (12.4%) (Khademi *et al*., 2020), Japan (9%) (Ikebe *et al*., 2021), and Spain (4.3%) (Villalón *et al*., 2021).

Resistance to fluoroquinolones, tetracyclines and vancomycin is not common but has been described (Kebede *et al*., 2021; Villalón *et al*., 2021; Barros 2021; Ubukata *et al*., 2020; Tatara *et al*., 2020). Similarly, most studies refer almost 100% susceptibility to penicillin (Andrina *et al*., 2020; Tsai *et al*., 2021; Grivea *et al*., 2020) but sub-clinical β-lactam tolerance has been described (Pichichero & Casey, 2007). Mutation in the *pbp2x* gene that encodes penicillin-binding protein 2x has been correlated with decreased susceptibility to β-lactams (Musser *et al*., 2020; Vannice *et al*., 2020). Interestingly, biofilm formation, entrance to epithelial cells and protection by other β-lactamase producing bacteria may also lead to penicillin ineffectiveness (Kebede *et al*., 2021) *in vivo*. In contrast to other data, GAS strains with high resistance rates to amoxicillin/clavulanic acid (89.5%) and cloxacillin (79%) have been reported in an Iranian study (Khademi *et al*., 2020). Wide spectrum cephalosporins are more frequently used for the treatment of *S. pyogenes* pharyngitis nowadays and many studies have concluded that oral cephalosporins are more efficient than oral penicillin (penicillin failure is estimated 2 times higher) (Khademi *et al*., 2020; Casey & Pichichero, 2004). However, cephalosporin resistance has been observed in Ethiopia and Japan (Kebede *et al*., 2021; Yanagihara *et al*., 2020).

MATERIALS AND METHODS

We conducted a retrospective observational study of *S. pyogenes* isolations from January 1, 2016 to December 31, 2021 in the AHEPA University Hospital, a 700-bed tertiary-care hospital in Thessaloniki in Northern Greece. The identification of the isolates and antimicrobial susceptibility testing were performed by the semi-automated VITEK2 system (bioMérieux, France). Interpretation of antimicrobial susceptibility results was done using EUCAST criteria.

RESULTS

During the six-year study period, 52 *S. pyogenes* isolates were recovered from 52 patients (24 pediatric patients and 28 adults). The samples included 14 pharyngeal, 12 blood, 9 wound, 9 ear, 5 surgical wound, 2 pleural fluid, and one tissue culture (*Table 2*). Antimicrobial susceptibility testing was performed for 49 isolates. All isolates were susceptible to penicillin, ampicillin, cefotaxime, ceftriaxone, linezolid, moxifloxacin, rifampicin, vancomycin, teicoplanin, and tigecycline. Erythromycin and clindamycin resistance rates were 20.4% (10/49) and 18.8% (9/48), respectively. Of the ten erythromycin-resistant isolates, only one expressed the M phenotype with resistance solely to erythromycin; the remaining nine were also resistant to clindamycin and, according to the test of the inducible clindamycin resistance performed by VITEK2, three belonged to the cMLSB and six to the iMLSB phenotype. Resistance rates to other antimicrobials were 40.8% (20/49) for tetracycline, 6.9% (2/29) for chloramphenicol, and 2% (1/49) for levofloxacin. The MIC_{50}, MIC_{90}, the MIC range and susceptibility rates for each antimicrobial are shown in *Table 3*.

DISCUSSION

Since the late 1990s there have been increased reports for resistant strains of GAS in Europe and other countries around the world. Greece is a country with increased rates of resistance to macrolides, but most data are regional from Central and Southern Greece.

Even though GAS is generally considered susceptible to β-lactams, isolates with reduced susceptibility or even resistance have emerged, mainly in China, and rare cases have also been reported from Mexico, India and Japan (Yu *et al*., 2020). Vannice *et al* have recently reported two almost identical strains with

Table 3 - *MIC_{50}, MIC_{90} and MIC range (mg/L) of antimicrobials against the S. pyogenes isolates. S: susceptible; I: susceptible increased exposure; R: resistant; NA: Not applicable.*

Antimicrobial	*MIC_{50}*	*MIC_{90}*	*MIC range*	*S No. (%)*	*I No. (%)*	*R No. (%)*
Ampicillin	≤ 0.25	≤ 0.25	≤ 0.25	29 (100%)	0 (0%)	0 (0%)
Benzylpenicillin	≤ 0.06	≤ 0.06	≤ 0.06 – 0.12	47 (100%)	0 (0%)	0 (0%)
Cefotaxime	≤ 0.12	≤ 0.12	≤ 0.12	NA	NA	NA
Ceftriaxone	≤ 0.12	≤ 0.12	≤ 0.12	NA	NA	NA
Chloramphenicol	2	4	2 – 4	27 (93.1%)	0 (0%)	2 (6.9%)
Clindamycin	≤ 0.25	≤ 0.25	≤ 0.25 – ≥ 1	39 (81.2%)	0 (0%)	9 (18.8%)
Erythromycin	≤ 0.12	≥ 8	≤ 0.12 – ≥ 8	39 (79.6%)	0 (0%)	10 (20.4%)
Levofloxacin	0.5	1	≤ 0.25 – 4	45 (91.8%)	3 (6.2%)	1(2%)
Linezolid	≤ 2	≤ 2	≤ 2	50 (100%)	0 (0%)	0 (0%)
Moxifloxacin	0.25	0.25	0.12 – 0.25	28 (100%)	0 (0%)	0 (0%)
Rifampicin	≤ 0.06	≤ 0.06	≤ 0.06	21 (100%)	0 (0%)	0 (0%)
Teicoplanin	≤ 0.12	≤ 0.12	≤ 0.12	20 (100%)	0 (0%)	0 (0%)
Tetracycline	≤ 0.25	≥ 16	≤ 0.25 – ≥ 16	29 (59.2%)	0 (0%)	20 (40.8%)
Tigecycline	≤ 0.06	≤ 0.06	≤ 0.06	28 (100%)	0 (0%)	0 (0%)
Vancomycin	0.25	0.5	≤0.12 – 0.5	50 (100%)	0 (0%)	0 (0%)

elevated MICs to ampicillin, amoxicillin and cefotaxime related to a novel pbp2x point mutation from patients under prior long-term β-lactam use (Vannice *et al*., 2020). It seems that like other streptococci, GBS and *S. pneumoniae*, GAS may have the capacity to escape antibiotic pressure through transformation of the peptidoglycan synthetic enzyme pbp2x. The authors of a recent study of 7025 genome sequences of GAS strains suggested that decreased β-lactam susceptibility was geographically widespread in strains with common emm gene subtypes (Musser *et al*., 2020). In our study, all isolates were susceptible to penicillin and β-lactams, in accordance with other studies from Greece.

Macrolides are recommended as an alternative treatment in case of allergy to β-lactams, but resistance rates have been increasingly reported in many countries, ranging widely from the lowest rate of 2% in Europe to the highest 90% in China (Rafei *et al*., 2022). Erythromycin resistance rate was 20.4% in our study (17.4% for pediatric patients and 23% for adults). Previous studies from Greece have reported rates that range from 11.9% to 38%, depending on the population tested (pediatric or adult), the type of infection (pharyngeal, non-pharyngeal, invasive, non-invasive), and the time period of the study (Grivea *et al*., 2020; Syrogiannopoulos *et al*., 2001; Petinaki *et al*., 2003; Stathi *et al*., 2008; Malli *et al*., 2010; Syrogiannopoulos *et al*., 2013; Michos *et al*., 2016; Beka *et al*., 2019). The highest rate (38%) was observed in pharyngeal specimens in 1998-2000 in central and southern Greece (Syrogiannopoulos *et al*., 2001). The lowest rate referred to invasive isolates, since non-invasive isolates are usually more resistant to erythromycin (Stathi *et al*., 2008). In our study, non-invasive represented 71.2% of the isolates. Our resistance rate of 20.4% is in accordance with more recent data (15.4%-20.4%) (Grivea *et al*., 2020; Michos *et al*., 2016; Beka *et al*., 2019).

In our study, most of the macrolide-resistant isolates belonged to either cMLSB or iMLSB phenotype. This is similar to other recent studies from Greece (Grivea *et al*., 2020; Michos *et al*., 2016) and many other European countries since the MLSB phenotype predominates over the M phenotype rate in the 2000s (Rafei *et al*., 2022).

Resistance to tetracycline was the predominant type of resistance in our strains, similar to a report from Spain (Villalón *et al*., 2021). Higher rates of resistance to tetracycline (40.8%) were observed in our study than in those referred from southern Greece (6.4%) (Michos *et al*., 2016), Ethiopia (14.3%) (Kebede *et al*., 2021), Iran (30.4%) (Khademi *et al*., 2020) and Brazil (31.8%) (Barros, 2021).

There are few reports with reference to quinolone resistance, mainly from Japan (11.1-14.3%), Ethiopia (7.1%), and Southern Greece (5.4%) (Michos *et al*., 2016). In our study, we observed a very low percentage of resistance (2%) to levofloxacin; all strains were susceptible to moxifloxacin.

Chloramphenicol resistance in our study presented a rate of 6.9%, similar to 7.1% from an Ethiopian report (Kebede *et al*., 2021) and higher than that referred from Southern Greece (2.9%) (Michos *et al*., 2016). Resistance to vancomycin has been reported in only one study from Ethiopia (35.5%) (Kebede *et al*., 2021). In our study, all isolates were susceptible to vancomycin, similar to another report from Greece (Michos *et al*., 2016).

While *S. pyogenes* commonly remains susceptible to

penicillin, treatment failure has been noticed and active surveillance for the emergence of penicillin resistance is of great clinical concern (Johnson & LaRock, 2021). Clindamycin serves as an alternative drug in case of β-lactam allergy, and a recent study has also pointed out the adjunctive role of clindamycin to β-lactam treatment of invasive GAS infections, leading to improved outcomes (Babiker *et al*., 2021). However, with the increasing rates of clindamycin resistance globally, physicians should be aware of the regional trends obtained by active surveillance (Johnson & LaRock, 2021).

Our study is a single-center study and the results do not necessarily reflect the overall national and international situation regarding the susceptibility profiles of *S. pyogenes*. Moreover, we did not use molecular epidemiology methods on our isolates since this was beyond the purpose of this work.

Different phenotypic antimicrobial patterns for *S. pyogenes* are observed per country or even among regions, and some are of considerable value for the appropriate treatment of GAS infections.

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