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Susceptibility patterns of *Streptococcus pyogenes* isolated in the Italian Hospital of Desio: a 20-year (2000-2019) retrospective study

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Summary

Background: *Streptococcus pyogenes* is a Gram-positive bacterium responsible for different infections, some of them serious and life-threatening. Increasing antimicrobial resistance trends have been observed in *S. pyogenes* isolates worldwide. In the present study, data of susceptibility patterns of *S. pyogenes* isolates were retrieved from 2000 to 2019 with the aim to observe the emergence of resistance and to guide clinicians in prescription appropriateness.

Materials and Methods: we collected a total of 4,958 unique *S. pyogenes* strains from positive samples, out of them 4,597 (93%) were from throat swabs, followed by vaginal swabs, skin swabs, blood cultures, and ear swabs. The median age of patients was 10 years (range 0-94 years).

Results: the analysis was performed on the data collected from throat samples and blood cultures. In the isolates from throat specimens, except β-lactams and glycopeptides (vancomycin) which presented 100% of susceptibility, and tetracycline which presented an average resistance rate of 26%, all the other antibiotics tested showed significant increasing resistance trends: clindamycin 13-23%, erythromycin 29-35%, and levofloxacin 5-12% ($p<0.01$). About susceptibilities of isolates from blood cultures, although the low number of isolates, we observed 98-100% of susceptibility.

Conclusions: although this work is single-center, national and regional active surveillance projects are needed to monitor antimicrobial resistance trends of *S. pyogenes*, and to help physicians in antibiotic prescription in subjects affected by acute or recurrent sore throats.

Key words: *Streptococcus pyogenes*; surveillance data; respiratory infections; throat swab; antibiotic.

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Introduction

Streptococcus pyogenes is a Gram-positive bacterium, facultative anaerobic, β-hemolytic, Lancefield Group A Streptococcus (GAS) [15]. *S. pyogenes* is one of the major pathogens responsible for a wide range of human diseases, ranging from pharyngitis, septicemia, impetigo, cellulitis, erysipelas, puerperal fever, and scarlet fever, to the severe toxic shock syndrome and necrotizing fasciitis. If untreated, the infections due to *S. pyogenes* can lead to autoimmune sequelae, such as glomerulonephritis and rheumatic fever [7,15,18,25,33,34,45]. The pathogen's ability to produce a diverse arsenal of virulence factors-including the M protein, streptolysins, and superantigens - facilitates immune evasion and tissue damage, contributing to its pathogenicity. The virulence factors include streptokinase, proteinases, esterase, hemolysins, DNases, M-protein, hyaluronidases, complement inhibitor, superoxide dismutase, and immunoglobulin-degrading enzymes. Moreover, the role of biofilm formation by *S. pyogenes* has gained increasing attention as a mechanism contributing to persistence and resistance to antibiotic treatment [4]. Humans are the main reservoir of *S. pyogenes*, and transmission mainly occurs by respiratory droplets and direct contact with skin lesions, and recently it has been reported also through foodborne [7,15,18,45]. It is estimated that *S. pyogenes* infections incidence is around 3 to 10 per 100,000 populations in developed countries, with a mortality rate of up to 20%, affecting both young and old subjects, in the case of invasive infection [30]. More recently, it has been reported from several countries in Europe an increasing trend in cases affected by invasive GAS infections after SARS-CoV 2 pandemic, particularly in the pediatric population [8]. Therefore, surveillance, prevention and targeted therapies of GAS infections are considered of strategic importance. The first line of choice in antibiotic therapy is the use of β-lactams, especially penicillin and amoxicillin, whereas cephalosporins are considered as an alternative therapy, with penicillin allergy [15,18,45]. However, the development of antimicrobial resistance has also been reported among streptococcal isolates worldwide, causing reduced susceptibility and treatment failures and severe threats to human health, as underlined by the World Health Organization (WHO) [43]. The mechanisms of antimicrobial resistance developed by streptococcus strains have been demonstrated to be different, such as enzymatic inactivation of antibiotics, modifications of the antimicrobial target, and preventing drug access to targets. Moreover, streptococcus strains are also recognized as biofilm producers, so causing persistent infections and multi-drugs resistance [3]. In the present work, because different resistance patterns for *S. pyogenes* have been reported according to geographical locations, we analyzed 20-year data (2000-2019) regarding the susceptibility patterns of *S. pyogenes* strains isolated in the Italian Hospital of Desio, with the aim to identify the emergence of antibiotic resistances during the study period and, re-viewing the literature, to provide useful information to guide clinicians in the best choice and rational use of antimicrobial treatments.

Materials and Methods

Study design and setting

This retrospective study analyzed antibiotic resistance patterns of *Streptococcus pyogenes* over a 20-year period (January 1, 2000 - December 31, 2019), using data from the Laboratory of Microbiology at Desio Hospital, Italy. If multiple isolates from the same subject showed identical resistance profiles, only the first isolate was included. Specimens containing organisms other than *S. pyogenes* were excluded.

Bacterial isolates and antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the VITEK® 1 and 2 systems (bioMérieux, Marcy l'Étoile, France) with AST-ST03 cards designed for streptococcal isolates. Resistance to nine antibiotics was evaluated: benzylpenicillin, amoxicillin/clavulanic acid, cefotaxime, cotrimoxazole (trimethoprim/sulfamethoxazole), clindamycin, erythromycin, tetracycline, levofloxacin, and vancomycin. Moreover, the macrolide resistance profiles for all erythromycin-resistant isolates was assessed to investigate constitutive and inducible Macrolide/Lincosamide/Streptogramin B resistance phenotype (MLSB), and macrolide only M phenotype. Interpretive criteria followed Clinical and Laboratory Standards Institute (CLSI) guidelines from 2000 to 2010 [10], and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria from June 2011 to December 2019 [13]. Bacterial identification was initially performed using the VITEK® systems and, from 2014 onward, by Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS) via the VITEK® MS system (bioMérieux). This system uses an AXIMA mass spectrometer (Shimadzu) linked to the SARAMIS v.4.12 database. Spectrum acquisition was managed using Launchpad software (v.2.9.3). *Escherichia coli* ATCC® 8739 served as a control strain throughout the study.

Statistical analysis

Data were analyzed using Stata Statistical Software (Release 16) [39]. Antimicrobial resistance trends were assessed using the chi-square test across four time intervals: 2000-2004, 2005-2009, 2010-2014, and 2015-2019. A p-value of less than 0.05 was considered statistically significant.

Results

In this study, we collected a total of 4,958 unique *S. pyogenes* strains from positive samples, that were divided in the following way: 4,597 (93%) from throat swabs, 192 (4%) from vaginal swabs, 107 (2%) from skin swabs, 38 (0.7%) from blood cultures, and 24 (0.3%) from ear swabs. The median age of patients was 10 years (range 0-94 years). Most of isolates were from females (51% compared to 49% males). Due to the greatest number of cases, we decided to conduct the analysis on the data from throat swabs and blood culture samples to compare the susceptibility patterns of *S. pyogenes* isolates.

Figure 1 shows the number of *S. pyogenes* isolates from positive throat swabs over the study period. It is possible to note the significant decreased number of positive samples between the first period (2000-2004, *n*=2,172) and the last period (2015-2019, *n*=489) ($p<0.05$). However, the percentages of positives were similar during the different intervals of time, from 13% (2,172/16,652) in the first period to 10% (489/4,753) in the last period ($p>0.05$), with an average value of 11% over the study time.

Figure 2 shows the relationship between age of subjects and the number of throat swab performed in the laboratory, and we observed that most of throat swabs was required between 0 and 14 years, followed by the range 26-46 years, highlighting that group 5-15 years old is the highest risk age group for *S. pyogenes* pharyngitis.

Figure 3 shows the *S. pyogenes* susceptibilities against the 9 antimicrobial drugs tested among all throat swab isolates enrolled in the study. From 2000-2004 to 2015-2019, except benzylpenicillin and the related amoxicillin + clavulanic acid, cefotaxime and vancomycin which did not show any resistance development (100% of susceptibility), and tetracycline which presented an average percentage of resistance of 26%, all the other antibiotics showed significant increasing resistance rates, clindamycin 13-23% and levofloxacin 6-12% (p value trend <0.01). Erythromycin resistance showed oscillations in the study period, it increased from 29% in the period 2000-2004 to 36% in 2010-2014 and decreased to 35% in the last period (p value trend <0.01). The evaluation of the resistance phenotypes revealed inducible MLSB resistance in 22 (3.5%) of the erythromycin-resistant isolates (*n*=640). Three of these isolates were resistant to erythromycin, but susceptible to clindamycin (M-type), suggesting the presence of an erythromycin efflux mechanism. Moreover, we observed the high resistance to trimethoprim-sulfamethoxazole antibiotic (96%), although it has been demonstrated that trimethoprim-sulfamethoxazole resistance is due to the technical limitations in laboratory methodology, particularly in the presence in media of high concentration of thymidine, that cause the inhibition of sulfur antibiotics [6].

Concerning *S. pyogenes* susceptibilities from isolates in blood cultures, although the low number of isolates, we observed 98-100% of susceptibility (Figure 4). Finally, the antibiotic resistance rates of *S. pyogenes* strains isolated from the other sources, such as from vaginal, skin, and ear swabs, presented similar results to those obtained from the throat swab samples, but the results were not statistically significant due to the small sample sizes ($p>0.05$).

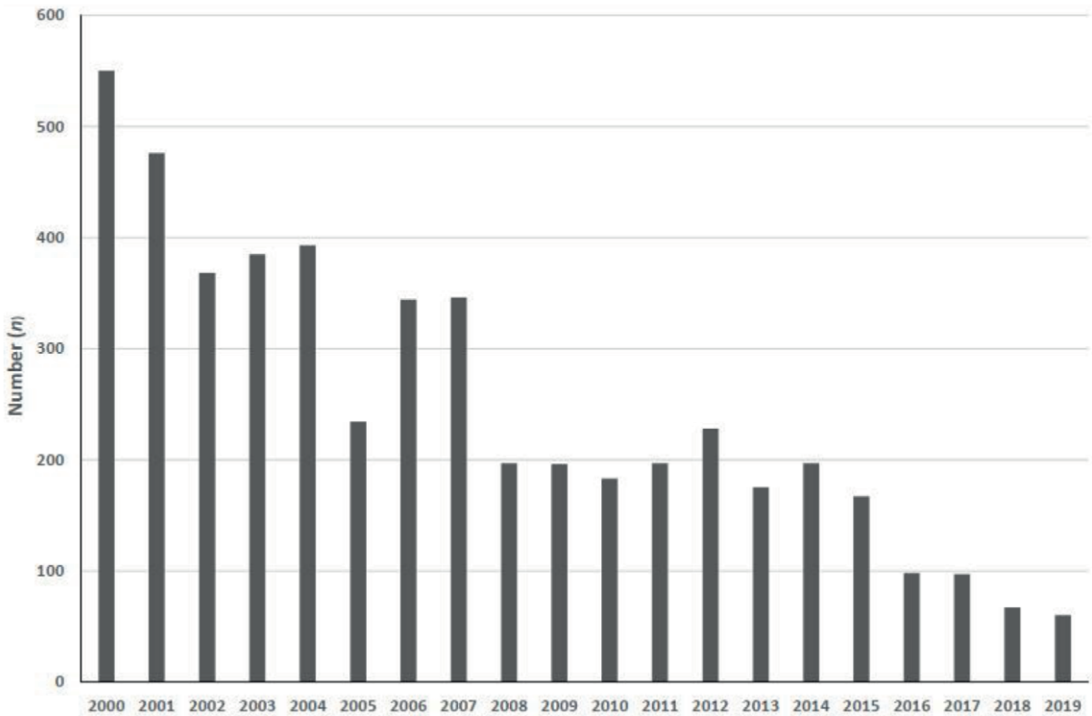

Figure 1. Number of *S. pyogenes* isolates from positive throat swabs during the period 2000-2019.

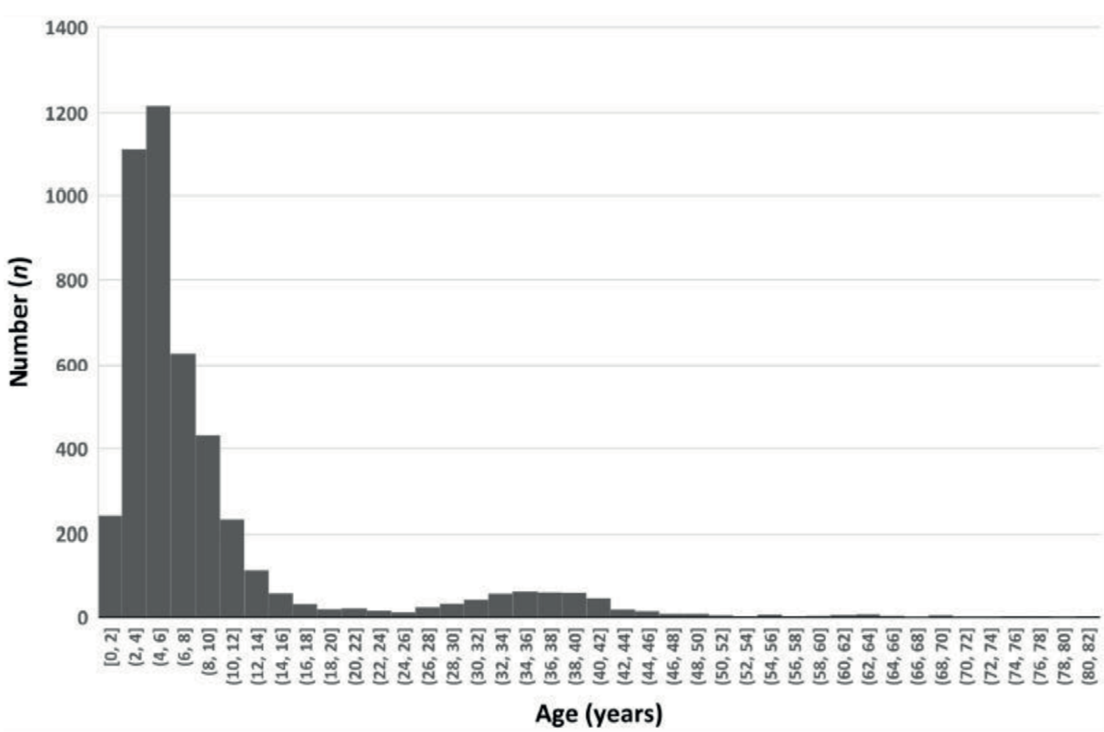

Figure 2. Number of throat swab prescriptions *versus* age's patient.

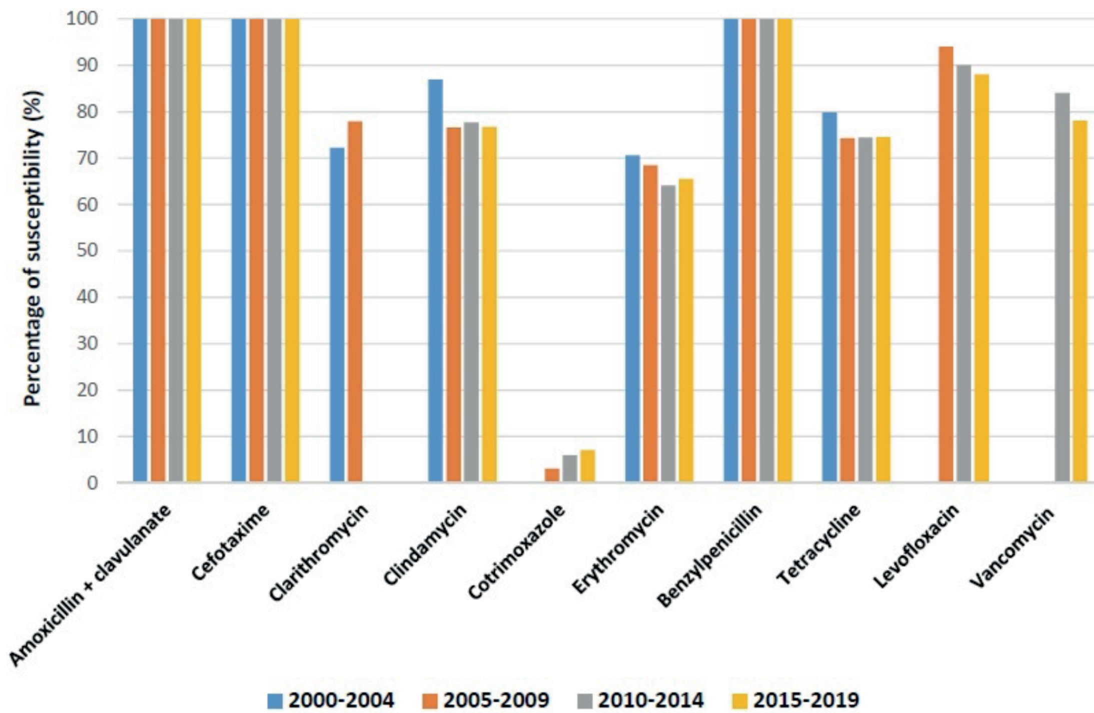

Figure 3. Antimicrobial trends of *S. pyogenes* isolated from throat swabs during the period 2000-2019.

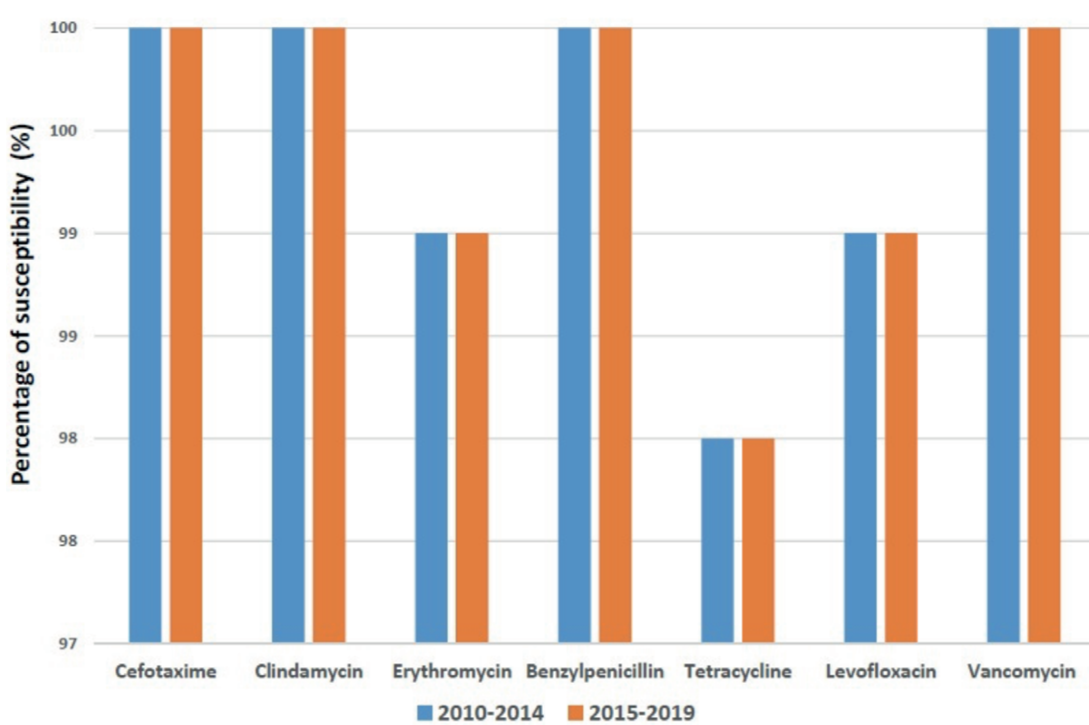

Figure 4. Susceptibilities trends of *S. pyogenes* isolates from blood cultures during the period 2000-2019.

Discussion

Antimicrobial resistance is one of the top 10 global human health problems [4]. In this paper, we conducted a retrospective analysis on data concerning the susceptibility patterns of *S. pyogenes* strains isolated over 20 years, with the aim to highlight the importance of an active antimicrobial surveillance. *S. pyogenes* does not belong to the bacteria under the European Antimicrobial Resistance Surveillance Network (EARS-Net) [12], but it causes more than 700 million of infections annually worldwide, with about 500,000 deaths due to the different virulence factors used by this microorganism [7,18,45]. Recently, after SARS-CoV-2 pandemic, there has also been an increased incidence of severe and invasive infections due to *S. pyogenes*, particularly in children less than 10-year of age, in different European countries (UK, Ireland, France, the Netherlands and Finland) and in the United States [8,44,9]. In 2022, Alcolea-Medina and coauthors reported that in London the increase of *S. pyogenes* infections were most probably due to decreased *S. pyogenes* exposure, particularly amongst children, and the high circulating levels of respiratory viruses, which may conduct to *S. pyogenes* infections [2]. Moreover, Italy is a country that presents high resistance rates among antibiotics, as documented by European and Italian surveillance reports [12,21]. Therefore, our work allowed us to describe the in-creased resistances over a long study period, in order to aid clinicians in the optimization of drug prescription appropriateness in cases of *S. pyogenes* infection.

Reviewing the literature, numerous studies on susceptibility trends are present, but it is important to note that the comparisons between them are difficult, because the type of population tested (pediatric or adult), the type of infection (invasive/non-invasive, pharyngeal/non-pharyngeal) and the study period are different. However, similarities and dis-similarities have been found. Moreover, although we used CLSI and EUCAST breakpoint guidelines due to the long study period, it has been reported an acceptable level of agreement between them, without altered antibiotic susceptibility interpretations [24].

S. pyogenes is generally considered universally susceptible to β-lactams, which inhibit cell wall synthesis and are used as first treatment choice by clinicians [18,42]. In our study, we observed a complete susceptibility to β-lactams (benzylpenicillin), and the related amoxicillin-clavulanic acid and the third-generation cephalosporin cefotaxime Although further research and confirmation are needed, it has been reported the presence of isolates with decreased susceptibility to β-lactams in China, Mexico and USA, most probably due to a pbp2x gene point mutation, which decreases the affinity of bacterial Penicillin-Binding Proteins (PBP) for β-lactams and could be a first step in development of resistance [41,45]. Among other streptococcal species, in Italy, from 2015 to 2022, the proportion of *Streptococcus pneumoniae* isolates resistant to penicillin remained substantially stable, while in Japan and also in North America *Streptococcus agalactiae* isolates have shown emerging reduced susceptibility to β-lactams due to amino acid substitutions in penicillin-binding proteins [21,19,27,41]. Macrolides, which inhibit protein synthesis, are the second treatment choice when a severe allergy to β-lactams is diagnosed in cases of pharyngitis due to *S. pyogenes*. On the other hand, in non-severe allergy cases, other β-lactams, *i.e.* cephalosporins, have better clinical activity than macrolides. Notably, a meta-analysis published in 2023 summarized macrolide resistance rates ranging from 10% to over 40% globally, with highest incidences reported in Asia and Southern Europe [18]. Regional surveillance further supports this variability: Portugal experienced a decline in *S. pyogenes* macrolide resistance from 2007 to 2013, driven in part by clonal shifts [38]; in Greece, Meletis and coauthors reported an erythromycin resistance rate of 20.4%, and in other studies the range was from 11.9% to 38% [32]; Abraham and Sistla reported an overall macrolide resistance rate of 53%, a value that is higher compared to other studies from India [1]; in Southern Hungary, Gajdacs and coauthors found a macrolide resistance rate ranging 23-40% [17]. These trends correspond well with the 33% erythromycin resistance observed in our Italian cohort.

Among the Lincosamides, which inhibit protein synthesis, clindamycin resistance increased 13-23% during the study period, a value which is similar to those from other European countries, such as Denmark (26%), Portugal (14.2-33.9%), France (26.3%), Spain (17.6%), but lower compared to that found in USA, 43.2% [18,42]. Among the tetracyclines, which inhibit protein synthesis, tetracycline is not routinely used to treat streptococcal infections, while doxycycline and minocycline are commonly used in clinical medicine, but these antibiotics present severe side effects, particularly in childhood and pregnant women. However, tetracyclines are extensively administered for the treatment and prophylaxis in animals around the world, favoring a high resistance among both Gram-negative and Gram-positive bacteria [18,29,42]. In our study, resistance to tetracycline did not increase over time, and the average value was 26%, similar to a re-port from Brazil (31.8%) and a report from Iran (30.4%) [5,26], lower than 40.8% from Greece and 46.3% from Poland [6], and higher compared to those referred from Norway (6.1%), Denmark (8%), southern Greece (6.4%), and Portugal (10.5%) [16,31]. The fluoroquinolones, which inhibit nucleic acid synthesis, are mainly used against bacteria belonging to the Streptococcus genus for the treatment of respiratory tract and urogenital tract infections. Reports on quinolones resistance of *S. pyogenes*, particularly levofloxacin, are rare. A reduced susceptibility to levofloxacin was firstly reported in Belgium during the period 2008-2010 (4.3-21.6%) [40]. After that, the quinolone resistance has been also described in Hungary (13.5%), Japan (11.1-14.3%), Ethiopia (7.1%), Greece (2%), and Germany (1.3%) [17,18,20,22]. In our study, we observed an average value of non-susceptibility of 11%. Lastly, among glycopeptides, which inhibit cell wall synthesis and are used in severe infections due to *S. pyogenes* [23], the data analysis on vancomycin resistance was per-formed during the two last study periods, 2010-2014 and 2015-2019, and resistances were not found. Resistance to vancomycin is extremely uncommon but it was reported in a study from Ethiopia (35.5%) [25]. The presence of van gene cassette has not been demonstrated in *S. pyogenes* [25]. Probably, biofilm formation is the cause of reduced susceptibility to vancomycin, underlying that further research is needed [23].

In our study, we also analyzed the susceptibility patterns of *S. pyogenes* strains isolated from blood cultures. Although the number of cases was low (*n*=38), the isolates from blood cultures did not present any development of antimicrobial resistances, most probably due to the absence of previous antimicrobial treatments. Concerning the low erythromycin resistance detected, our findings are consistent with data from the National Surveillance in Spain (2007-2020), which similarly reported low and progressively declining erythromycin resistance among invasive *S. pyogenes* isolates, most probably due to a reduced macrolide consumption and shifts in circulating clones, resulting in a decreased prevalence of strains harboring macrolide-resistance genes [42]. Comparable trends have also been described in Italy, where an 11-year surveillance study documented changes in *emm* types, virulence profiles, and antibiotic resistance patterns among invasive *S. pyogenes* isolates, highlighting the dynamic interplay between clonal replacement and resistance evolution [11]. Moreover, the introduction of *Streptococcus* Group A detection rapid tests (detectable in our work in the significant decreased number of throat swab cultures performed in the laboratory from 2000 (*n*=3,989) to 2019 (*n*=602)) was an important advance, allowing the diagnosis of *S. pyogenes* throat infection in a few minutes [36]. However, the presence of false positive and false-negative results using this methodology, and the difficulties of physicians to distinguish between sore throats of viral or bacterial origin, determined a wrong use of antibiotics, therefore increasing the possibility of developing resistances, as recently described [37].

Our study presents some limitations that need to be not neglected: i) the work was retrospective and performed in a single center, and a larger number of subjects is needed to confirm our results; ii) the study period encompassed isolates collected between 2000 and 2019, which is relatively old and may not accurately represent the current resistance patterns circulating among GAS; iii) the low numbers of isolates from skin swabs and ear swabs; iv) the low number of isolates from blood cultures with the aim to compare the results to those from non-invasive throat isolates; v) the absence of clinical correlation of reported *in vitro* resistance and clinical resistance (treatment failure); vi) the absence of genetic data, but the implementation of whole-genome sequencing technology could allow to characterize the dynamics and features of strains associated with virulence factor distributions, diffusion of resistance, and putative vaccine targets in many geographic areas.

The COVID-19 pandemic has indirectly influenced the epidemiology of GAS and antibiotic resistance patterns by disrupting routine healthcare, altering antibiotic prescribing practices, and affecting transmission dynamics [28]. In fact, in a post-COVID study, erythromycin resistance among GAS isolates increased markedly from 6% in 2020 to 25% during 2021-2022, with 13% of isolates overall showing erythromycin resistance [35]. Similarly, data from the US CDC Active Bacterial Core Surveillance program reported increases in erythromycin- and clindamycin-resistant invasive GAS strains from 11.9% to 24.7% and from 8.9% to 23.8%, respectively [14]. These observations highlight the necessity of integrating antimicrobial stewardship with active regional and national surveillance. Such integration can support the monitoring of antimicrobial resistance trends and improve the appropriateness of antibiotic prescriptions - not only for human health but also for animal livestock, food production, and agriculture, which are interconnected.

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