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# A rapid increase in macrolide resistance in *Streptococcus pyogenes* isolated in Poland during 1996–2002

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***Objectives*: The aim of this study was to investigate Polish clinical isolates of *Streptococcus pyogenes* collected during a 7 year period using phenotypic and genotypic techniques.**

***Methods*: A total of 816 isolates of *S. pyogenes* recovered from 33 medical centres in Poland were tested for their susceptibility to various antimicrobial agents. Erythromycin-resistant isolates were analysed by PFGE, multilocus sequence typing and *emm* typing methods.**

***Results*: The tetracycline resistance rate was high (43%) among all *S. pyogenes* strains. Ninety-eight (12%) isolates were resistant to erythromycin. A low prevalence of the M phenotype (5.1%) associated with the presence of the *mef*(A) gene was found. All the isolates of the $iMLS_B$ phenotype harboured the *erm*(TR) gene. Out of the $cMLS_B$ isolates, 71.4% and 28.6% carried *erm*(TR) and *erm*(B), respectively. All isolates with *erm*(B) were resistant to telithromycin. PFGE analysis discerned 13 different patterns, A–N, with two predominant PFGE profiles—A (41 isolates) and B (25 isolates)—that in multilocus sequence typing corresponded, respectively, to a novel sequence type (ST) 367 and ST63. Overall, the representatives of these clones accounted for >90% of isolates of the $iMLS_B$ phenotype.**

***Conclusions*: A significant increase in erythromycin resistance was observed among clinical *S. pyogenes* collected in Poland over a 7 year period driven by the spread of two epidemic clones.**

Keywords: *S. pyogenes*, erythromycin resistance, phenotypes, genotypes

## Introduction

Since the 1940s, penicillin has been the treatment of choice for *Streptococcus pyogenes* infections, whereas erythromycin and clindamycin are usually recommended as alternative antibiotics. The first erythromycin-resistant strain of *Streptococcus* was described in 1959 in the UK and since then in many countries.[1] Whereas data regarding the prevalence of *S. pyogenes* resistance to macrolides have been reported worldwide, the problem in Poland has remained unstudied until now.

The aim of this study was to investigate the susceptibility patterns of Polish clinical isolates of *S. pyogenes*, with particular emphasis on macrolide resistance and the underlying genetic determinants of this resistance. The clonal structure of macrolide-resistant isolates was studied by PFGE of *Sma* I-restricted bacterial DNA as well as multilocus sequence typing (MLST) and *emm* typing of selected strains.

## Materials and methods

### *Bacterial isolates*

Eight hundred and sixteen isolates of *S. pyogenes* were collected during 1996–2002 at 33 medical centres in Poland. The number of strains collected in the periods 1996–1997, 1998–1999 and 2000–2002 were 266, 258 and 292, respectively. The isolates were recovered from throat swabs ($n=438$), pus ($n=331$), blood ($n=17$), sputum ($n=8$) and other sources ($n=22$). Of these, 361 isolates were obtained from children and 455 from adult patients. *S. pyogenes* were identified by standard procedure using a commercially available agglutination kit (Streptex, Murex Biotech Limited, UK).

### *Determination of MICs and erythromycin resistance phenotypes*

MICs were determined according to the NCCLS guidelines[2] by the standard microdilution method. *Streptococcus pneumoniae* ATCC

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49619 was used as a quality control strain. The MIC breakpoints were interpreted according to the NCCLS.[2] Breakpoints for spiramycin were those proposed by the French Society for Microbiology.[3] For all strains, susceptibility to the following antimicrobials was tested: penicillin G, erythromycin, tetracycline (Sigma-Aldrich, Steinheim, Germany) and clindamycin (Pharmacia Upjohn, Inc., Kalamazoo, MI, USA). Erythromycin-resistant strains (MIC≥1 mg/L) were additionally tested for susceptibility to clarithromycin (Abbott Laboratories Chicago, IL, USA), roxithromycin (Roussel Uclaf, Paris, France), azithromycin (Pliva Kraków, Poland), spiramycin, (Rhone-Poulenc Rorer, Collegeville, PA, USA), telithromycin, quinupristin/dalfopristin (Aventis Pharma, Romainville, France), linezolid (Pharmacia & Upjohn, Inc., Kalamazoo, MI, USA) and moxifloxacin (Bayer AG, Wuppertal, Germany). All erythromycin-resistant isolates were assigned to their particular phenotypes, such as inducible $MLS_B$ ($iMLS_B$), constitutive $MLS_B$ ($cMLS_B$) and efflux-mediated resistance (M phenotype) on the basis of the double erythromycin–clindamycin disc test.[4]

### *Detection of erythromycin resistance genes*

PCR detection of *erm*(B) and *mef*(A) genes was carried out as reported by Sutcliffe *et al.*[5]; the *erm*(TR) gene was detected with primers described by Kataja *et al.*[4]

### *PFGE analysis, MLST and emm typing*

Chromosomal DNA of erythromycin-resistant isolates was digested with *Sma*I restriction enzyme (MBI Fermentas, Lithuania) and analysed by PFGE, as described elsewhere.[6] PFGE patterns were analysed according to the criteria proposed by Tenover *et al.*[7] MLST was performed on representatives of all PFGE types and subtypes, following the method established by Enright *et al.*[8]; particular allele numbers and sequence types (STs) were identified using the MLST database (www.mlst.net). *emm* types for the isolates characterized by MLST were determined according to the recommendations of the Division of Bacterial and Mycotic Diseases, Centers for Disease Control and Prevention, *S. pyogenes emm* sequence database (http://www.cdc.gov/ncidod/biotech/strep/doc.htm).

### *Statistical analysis*

Comparison of frequencies between groups was performed using $\chi^2$ analysis. A $P$ value of <0.05 was considered statistically significant.

## Results and discussion

All 816 clinical *S. pyogenes* isolates examined in our study were highly susceptible to penicillin G. The highest rate of resistance was found for tetracycline (43%) and the rate was higher among isolates from adults (62%). These results are similar to those observed by Jasir *et al.*[9] in Iran and once again indicate that tetracycline is not an appropriate choice for empirical therapy of *S. pyogenes* infections.

Several European countries reported an increase in erythromycin resistance in *S. pyogenes* at the beginning of the 1990s.[1] In our study, altogether 98 isolates (12.0%) were resistant to erythromycin. The proportion of these isolates in adults was higher (64%) than in children. The frequency of erythromycin-resistant strains showed a rising trend over the years, from 1.8% in 1996–1997, to 12% in 1998–1999, to 25.1% in 2000–2002. The $P$ values between 1.8% and 12%, 12% and 25.1% and 1.8% and 25.1% were 0.001, 0.01 and 0.001, respectively, showing a statistical significance. All erythromycin-susceptible isolates were also susceptible to clindamycin (MICs, 0.003–0.25 mg/L). Among the 98 erythromycin-resistant isolates, 65 (66.6%) exhibited the $iMLS_B$ and 28 (28.2%) the $cMLS_B$ phenotype (Table 1). All the $iMLS_B$ phenotypes harboured the *erm*(TR) gene. In the more diverse group of $cMLS_B$ isolates, 20 (71.4%) and eight (28.6%) contained *erm*(TR) and *erm*(B), respectively. Overall, 85 isolates (86.7%) showed the presence of *erm*(TR). Such a high prevalence of the *erm*(TR) isolate among macrolide-resistant *S. pyogenes* in Poland is unusual compared with other countries.[4,10–12] The M phenotype occurred in only five isolates (5.1%) and all possessed the *mef*(A) gene. Similarly, a low prevalence of the M phenotype (10%) was found in a study conducted in Berlin.[10] However, other investigators have demonstrated that the M phenotype accounted for >80%.[1] In our study, all $iMLS_B$ and M-phenotype isolates were susceptible to clindamycin

**Table 1.** Susceptibility patterns of 98 isolates of erythromycin-resistant *S. pyogenes* according to macrolide-resistance phenotypes

| Antibiotic | iMLS ($n=65$) | | | | cMLS ($n=28$) | | | | M phenotype ($n=5$) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MIC range (mg/L) | $MIC_{50}$ | $MIC_{90}$ | % R | MIC range (mg/L) | $MIC_{50}$ | $MIC_{90}$ | % R | MIC range (mg/L) | $MIC_{50}$ | $MIC_{90}$ | % R |
| Erythromycin | 1–64 | 1 | 4 | 100 | 2–>128 | 8 | >128 | 100 | 1–16 | 4 | 8 | 100 |
| Clarithromycin | 0.5–128 | 1 | 2 | 100 | 2–>128 | 16 | >128 | 100 | 2–8 | 2 | 2 | 100 |
| Roxithromycin | 1–>128 | 4 | 16 | 100 | 4–>128 | 32 | >128 | 100 | 2–8 | 4 | 4 | 100 |
| Azithromycin | 1–128 | 1 | 2 | 100 | 4–>128 | 32 | >128 | 100 | 2–8 | 4 | 4 | 100 |
| Spiramycin | 0.25–128 | 1 | 4 | 29.2 | 16–>128 | 16 | >128 | 100 | 0.12–1 | 0.25 | 0.5 | 0 |
| Clindamycin | 0.03–0.25 | 0.12 | 0.25 | 0 | 2–>128 | 64 | >128 | 100 | 0.03–0.12 | 0.12 | 0.06 | 0 |
| Telithromycin | 0.008–0.25 | 0.03 | 0.06 | 0 | 0.03–32 | 0.12 | 4 | 28.5 | 0.12–0.25 | 0.12 | 0.12 | 0 |
| Q/D | 0.12–1 | 0.5 | 1 | 0 | 0.12–1 | 0.5 | 1 | 0 | 0.25–1 | 1 | 1 | 0 |
| Moxifloxacin | 0.12–0.25 | 0.12 | 0.25 | 0 | 0.03–0.25 | 0.12 | 0.12 | 0 | 0.06–0.12 | 0.12 | 0.12 | 0 |
| Linezolid | 0.25–1 | 0.5 | 1 | 0 | 0.5–1 | 1 | 1 | 0 | 0.5–1 | 1 | 1 | 0 |
| Tetracycline | 8–64 | 16 | 32 | 100 | 0.12–128 | 2 | 64 | 50 | 0.12–2 | 0.25 | 0.5 | 0 |

Q/D, quinupristin/dalfopristin; % R, percentage of resistant isolates.

**Table 2.** Profile PFGE, *emm* types and MLST of erythromycin-resistant *S. pyogenes* isolates

| PFGE profile | Number of strains | Macrolide resistance | | *emm*[a] | MLST allele[a] | | | | | | | ST |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | phenotype | genotype | | *gki* | *gtr* | *murL* | *mutS* | *recP* | *xpt* | *yqiL* | |
| A1 | 30 | i | *erm*(TR) | 44/61 | 4 | 2 | 3 | 11 | 17 | 3 | 61[b] | **367** |
| A2 | 2 | i | *erm*(TR) | 44/61 | 4 | 2 | 3 | 11 | 17 | 3 | 61[b] | **367** |
| A3 | 2 | i | *erm*(TR) | 44/61 | 4 | 2 | 3 | 11 | 17 | 3 | 61[b] | **367** |
| A4 | 2 | i | *erm*(TR) | 44/61 | 4 | 2 | 3 | 11 | 17 | 3 | 61[b] | **367** |
| A5 | 5 | i | *erm*(TR) | 44/61 | 4 | 2 | 3 | 11 | 17 | 3 | 61[b] | **367** |
| B1 | 20 | i | *erm*(TR) | 77 | 13 | 6 | 2 | 3 | 23 | 3 | 11 | 63 |
| B2 | 1 | i | *erm*(TR) | 77 | 13 | 6 | 2 | 3 | 23 | 3 | 11 | 63 |
| B3 | 1 | i | *erm*(TR) | 77 | 13 | 6 | 2 | 3 | 23 | 3 | 11 | 63 |
| B4 | 3 | i | *erm*(TR) | 77 | 13 | 6 | 2 | 3 | 23 | 3 | 11 | 63 |
| F1 | 1 | M | *mef*(A) | 77 | 13 | 6 | 2 | 3 | 23 | 3 | 11 | 63 |
| G1 | 1 | i | *erm*(TR) | 77 | 13 | 6 | 2 | 3 | 23 | 3 | 11 | 63 |
| H1 | 2 | i | *erm*(TR) | 77 | 13 | 6 | 2 | 3 | 23 | 3 | 11 | 63 |
| C1 | 1 | c | *erm*(B) | 1 | 4 | 3 | 4 | 4 | 4 | 2 | 4 | 28 |
| C2 | 1 | c | *erm*(TR) | 1 | 4 | 3 | 4 | 4 | 4 | 2 | 4 | 28 |
| C3 | 1 | c | *erm*(TR) | 1 | 4 | 3 | 4 | 4 | 4 | 2 | 4 | 28 |
| D1 | 1 | c | *erm*(B) | 12 | 5 | 2 | 2 | 6 | 6 | 2 | 2 | 36 |
| D2 | 2 | c | *erm*(B) | 12 | 5 | 2 | 2 | 6 | 6 | 2 | 2 | 36 |
| D3 | 3 | c | *erm*(B) | 12 | 5 | 2 | 2 | 6 | 6 | 2 | 2 | 36 |
| nt | 3 | M | *mef*(A) | 12 | 5 | 2 | 2 | 6 | 6 | 2 | 2 | 36 |
| I1 | 3 | c | *erm*(TR) | 12 | 93[b] | 2 | 2 | 6 | 6 | 2 | 2 | **366** |
| E1 | 6 | c | *erm*(TR) | 75 | 11 | 2 | 1 | 3 | 50 | 8 | 7 | 150 |
| E2 | 3 | c | *erm*(TR) | 75 | 11 | 2 | 1 | 3 | 50 | 8 | 7 | 150 |
| K1 | 1 | c | *erm*(B) | 22 | 9 | 8 | 1 | 1 | 1 | 3 | 4 | 46 |
| L1 | 1 | i | *erm*(TR) | 60 | 11 | 6 | 22 | 7 | 9 | 2 | 17 | 53 |
| M1 | 1 | i | *erm*(TR) | 28 | 11 | 6 | 14 | 5 | 9 | 44 | 19 | 244 |
| N1 | 1 | M | *mef*(A) | 28 | 11 | 6 | 14 | 5 | 9 | 17 | 19 | 52 |

i, inducible $MLS_B$ phenotype; c, constitutive $MLS_B$ phenotype; M, efflux-mediated resistance; underlined, locus variant; bold type, new ST.
[a]*emm* types and MLST of selected isolates.
[b]New allele.

and telithromycin. In contrast, all $cMLS_B$ isolates were resistant to clindamycin. In the case of ketolides, we found eight (8.1%) *erm*(B)-positive $cMLS_B$ isolates that were resistant to telithromycin (Table 1). The data presented here, which confirmed that the isolates with the *erm*(B) gene were resistant to telithromycin, are in accordance with studies from Spain[11] and from other European countries.[12] In the present study, we determined the susceptibilities of macrolide-resistant *S. pyogenes* to antibiotics belonging to various groups. All tested isolates were fully susceptibility to linezolid, moxifloxacin and quinupristin/dalfopristin. These agents demonstrated good *in vitro* activity independent of the macrolide resistance phenotype present (Table 1). Hence these antibiotics could be used as alternatives for the treatment of *S. pyogenes* infections in selected cases.

Until now, no data have been available on the genetic diversity of Polish *S. pyogenes* isolates resistant to macrolides. PFGE analysis was performed on 95 of the 98 macrolide-resistant strains (three M-phenotype isolates were not typeable). Altogether, 13 different PFGE patterns, designated A–N, were discerned among the isolates (Table 2), with two predominant PFGE profiles A ($n = 41$) and B ($n = 25$). Among the type A and B isolates, five (A1–A5) and four (B1–B4) PFGE subtypes were found, respectively. Whereas all the 41 isolates of the PFGE subtypes A1–A51 showed the $iMLS_B$ phenotype, six isolates of the PFGE type B1 had the $cMLS_B$ phenotype and 19 (B1-B4) were $iMLS_B$. All the isolates belonging to the A and B clones harboured the *erm*(TR) gene (Table 2) and were resistant to tetracycline. Thirty-three (80%) isolates of the A type were recovered from wounds, whereas the majority of the B type (76%) were from throat samples. Overall, the isolates belonging to clones A and B accounted for almost 90% of isolates of the $iMLS_B$ phenotype. It can be concluded, therefore, that *S. pyogenes* of the $iMLS_B$ phenotype in Poland are highly clonal. In contrast, the $cMLS_B$ isolates showed a polyclonal nature (six different PFGE types).

The representatives of all PFGE types and subtypes were further subjected to MLST analysis and *emm* typing, resulting in 10 different STs and eight *emm* types (Table 2). The majority of STs found in this study, in particular: ST63 (characteristic of all the subtype B1–B4 isolates as well as isolates of types F, G, H); ST28 (subtypes C1–C3); ST36 (subtypes D1–D3 and three non-typeable isolates); ST46 (type K) and ST52 (type N) have been previously found among erythromycin-resistant *S. pyogenes* in Germany.[13] In contrast, the main clone of PFGE subtypes A1–5 represented a novel ST367 (*emm* type 44/61). This clone, in which resistance developed presumably locally, constitutes a single local variant of ST25, isolated in the 1950s and 1970 in Northern and Central America.[8] Erythromycin and tetracycline resistance may, in this case, represent a strong selective advantage, which drives successful spread of the new clone under antibiotic pressure.

In summary, the present study demonstrates the alarming increase in macrolide resistance in *S. pyogenes* in Poland over

the few past years. A significant part of this increase can be attributed to the successful dissemination of two epidemic *S. pyogenes* clones, each involved in a different type of infection. Unfortunately, these clones, and thus the majority of macrolide-resistant strains possess the $MLS_B$ phenotype conferring resistance to all macrolides, lincosamides and streptogramin B. Moreover, these clones are resistant to tetracycline. Resistance to a novel ketolide, telithromycin, albeit presently low, may grow under increased selective pressure as strains with this characteristic circulate within the population. Although several therapeutic options still remain for the treatment of *S. pyogenes* infections, judicious use of all available antimicrobials and continued monitoring of susceptibility of this pathogen are critical for the future.

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## References

**1.** Leclercq, R. (2002). Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. *Clinical Infectious Diseases* **34**, 482–92.

**2.** National Committee for Clinical Laboratory Standards. (2004). *Performance Standards for Antimicrobial Susceptibility Testing; Fourteenth Informational Supplement M100-S14*. NCCLS, Wayne, PA, USA.

**3.** Report of the Comité de l'Antibiogramme de la Société Française de Microbiologie. (1996). Statement 1996 CA-SFM. Zone sizes and MIC breakpoints for non-fastidious organisms. *Clinical Microbiology and Infection* **2**, *Suppl. 1*, 46–9.

**4.** Kataja, J., Huovinen, P., Skurnik, M. *et al.* (1999). Erythromycin resistance genes in group A streptococci in Finland. *Antimicrobial Agents and Chemotherapy* **43**, 48–52.

**5.** Sutcliffe, J., Grebe, T., Tait-Kamradt, A. *et al.* (1996). Detection of erythromycin-resistant determinants by PCR. *Antimicrobial Agents and Chemotherapy* **40**, 2562–6.

**6.** Stanley, J., Linton, D., Desai, M. *et al.* (1995). Molecular subtyping of prevalent M serotypes of *Streptococcus pyogenes* causing invasive disease. *Journal of Clinical Microbiology* **33**, 2850–5.

**7.** Tenover, F. C., Arbeit, R. D., Goering, R. *et al.* (1995). Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *Journal of Clinical Microbiology* **33**, 2233–9.

**8.** Enright, M. C., Spratt, B. G., Kalia, A. *et al.* (2001). Multilocus sequence typing of *Streptococcus pyogenes* and the relationships between *emm* type and clone. *Infection and Immunity* **69**, 2416–7.

**9.** Jasir, A., Tanna, A., Noorani, A. *et al.* (2000). High rate of tetracycline resistance in *Streptococcus pyogenes* in Iran: an epidemiological study. *Journal of Clinical Microbiology* **38**, 2103–7.

**10.** Arvand, M., Hoeck, M., Hahn, H. *et al.* (2000). Antimicrobial resistance in *Streptococcus pyogenes* isolates in Berlin. *Journal of Antimicrobial Chemotherapy* **46**, 621–3.

**11.** Morosini, M. I., Cantón, R., Loza, E. *et al.* (2003). *Streptococcus pyogenes* isolates with characterized macrolide resistance mechanisms in Spain: *in vitro* activities of telithromycin and cethromycin. *Journal of Antimicrobial Chemotherapy* **52**, 50–5.

**12.** Nagai, K., Appelbaum, P. C., Davies, T. A. *et al.* (2002). Susceptibility to telithromycin in 1,011 *Streptococcus pyogenes* isolates from 10 Central and Eastern European Countries. *Antimicrobial Agents and Chemotherapy* **46**, 546–9.

**13.** Reinert, R. R., Lütticken, R., Sutcliffe, J. A. *et al.* (2004). Clonal relatedness of erythromycin-resistant *Streptococcus pyogenes* isolates in Germany. *Antimicrobial Agents and Chemotherapy* **48**, 1369–73.