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***mef(A)* is the predominant macrolide resistance determinant in *Streptococcus pneumoniae* and *Streptococcus pyogenes* in Germany**

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ABSTRACT

In this study, macrolide-resistant *Streptococcus pneumoniae* and *Streptococcus pyogenes* isolates from Germany were carefully characterised by susceptibility testing, phenotyping, polymerase chain reaction (PCR) and sequencing of macrolides resistance genes, and multilocus sequence typing (MLST). Of 2045 *S. pneumoniae* and 352 *S. pyogenes* isolates, 437 (21.4%) and 29 (8.2%), respectively, were found to be macrolide-resistant. Among the *S. pneumoniae* isolates, the most prevalent resistance marker was *mef(A)* (57.7%) followed by *erm(B)* (27.0%) and *mef(E)* (11.2%). Of note, the dual resistance mechanism *mef(E)+erm(B)* was found in a relatively high proportion (4.1%) of pneumococcal isolates. Among the *S. pyogenes* isolates, 31.0% carried *mef(A)*, 34.5% *erm(B)* and 13.8% *erm(A)*. Dissemination of a single clone [*mef(A)*-positive England¹⁴⁻⁹] has significantly contributed to the emergence of macrolide resistance among pneumococci in Germany.

1. Introduction

Streptococcus pneumoniae is a major cause of many childhood diseases such as respiratory tract infections, acute otitis media, community-acquired pneumonia, meningitis and sepsis [1]. *Streptococcus pyogenes* (Group A *Streptococcus*) is responsible for the majority of cases of pharyngitis but can also cause much more severe infections such as necrotizing fasciitis and toxic shock syndrome [2].

Resistance of *S. pneumoniae* and *S. pyogenes* to macrolides has become an increasing problem worldwide owing to spread of the resistance genes *mef*(A), subclasses *mef*(A) and *mef*(E), as well as *erm*(A) and *erm*(B) [3,4]. Streptococci can acquire macrolide resistance through various mechanisms, for example efflux and methylation. The efflux mechanism consists of a pump encoded by the *mef* genes *mef*(A) and *mef*(E), and an ATP-binding cassette protein encoded by the *mel* gene, which reduces the intracellular antibiotic concentration to subtoxic levels [5,6], and is associated with the M-phenotype [7–11]. The sequences of the *mef*(A) and *mef*(E) genes are very similar but they are located on different genetic elements. *mef*(A) is located on the transposon Tn1207.1, whereas *mef*(E) is located on the so-called MEGA (macrolide efflux gene assembly) element. The second macrolide resistance mechanism is a 23S rRNA methylase that mediates ribosomal modification of the macrolide-binding site and is encoded by the *erm* genes located on conjugative transposons such as Tn1545 and Tn2010. Is associated with the MLS_B phenotype, which confers multidrug resistance to 14-, 15- and 16-membered ring macrolides,

lincosamides and streptogramin B [5,12,13]. *erm*(B) has been described in *S. pneumoniae*, and *erm*(B) and *erm*(A) in *S. pyogenes* [14].

Other mechanisms of macrolide resistance include mutations in domain V of 23S rRNA as well as modifications of ribosomal proteins L4 and L22, which are located in the direct vicinity of the macrolide-binding site [8–10,15].

The purpose of this study was (i) to analyse the macrolide resistance gene distribution in a large collection of clinical streptococcal isolates and (ii) to analyse the clonal distribution of these isolates and their contribution to the recent emergence of macrolide resistance in Germany.

2. Material and methods

2.1. Study design and bacterial isolates

Invasive streptococcal isolates are sent to the German National Reference Center for Streptococci (GNRCS) (Aachen, Germany) as part of active prospective surveillance by more than 300 microbiology laboratories throughout Germany [16]. Non-invasive isolates are sent to GNRCS as part of several local surveillance studies. Between January 2005 and December 2006, GNRCS received a total of 2045 (1887 invasive and 158 non-invasive) *S. pneumoniae* and 352 (227 invasive and 125 non-invasive) *S. pyogenes* isolates. The present study focuses on characterisation of 437 macrolide-resistant *S. pneumoniae* [minimal inhibitory

concentration (MIC) for clarithromycin $>1 \mu\text{g/mL}$] and 29 macrolide-resistant *S. pyogenes* isolates.

Strain identification by local laboratories was confirmed at GNRCS for *S. pneumoniae* by optochin susceptibility and bile solubility testing. Optochin susceptibility testing was carried out in a 5% CO_2 atmosphere on sheep blood agar. For bile solubility testing, a bacterial suspension in 1 mL of 0.85% NaCl (McFarland standard 1.0) with four drops of 10% sodium desoxycholate was prepared. Following incubation for 2 h at 35 °C, complete lysis was taken as a positive result.

Streptococcus pyogenes isolates were identified by β -haemolysis on sheep blood agar, Lancefield grouping using a commercially available agglutination technique (Slidex® Strepto Kit; bioMérieux, Marcy-l'Étoile, France) and the pyrrolidonyl arylamidase test [17].

2.2. Susceptibility testing

All strains were tested for antibiotic MICs using the broth microdilution method as recommended by the Clinical and Laboratory Standards Institute (CLSI) [18]. The following breakpoints ($\mu\text{g/mL}$) used were for susceptible, intermediate and resistant: clarithromycin, ≤ 0.25 , 0.5 and ≥ 1 ; penicillin (parenteral), ≤ 0.06 , – and ≥ 0.12 [meningitis, isolates from cerebrospinal fluid (CSF)]/ ≤ 2 , 4 and ≥ 8 (non-meningitis, isolates from sources other than CSF); clindamycin, ≤ 0.25 , 0.5 and ≥ 1 ; tetracycline, ≤ 2 , 4 and ≥ 8 ; and levofloxacin ≤ 2 , 4 and ≥ 8 .

Microtitre plates (Sensititre NLMMCS10; TREK Diagnostic Systems Ltd., East Grinstead, UK) contained penicillin G, cefotaxime, clarithromycin, clindamycin, tetracycline and levofloxacin with cation-adjusted Mueller–Hinton broth (Oxoid, Wesel, Germany) and 5% lysed horse blood. The final inoculum was 5×10^5 colony-forming units/mL. Incubation was at 37 °C for 24 h in ambient air. *Streptococcus pneumoniae* ATCC 49619 was used as a quality control strain. For interpretation, CLSI criteria were applied [18]. In this study, co-resistance is defined as a macrolide-resistant strain that shows resistance to one of the other antibiotics also tested in this study. To define the macrolide resistance phenotype, disks of erythromycin (15 µg), clindamycin (2 µg) and rokitamycin (30 µg) (Oxoid Ltd., Basingstoke, UK) were placed 10–15 mm apart on Mueller–Hinton agar with 5% sheep blood (Oxoid). For phenotyping of *S. pyogenes* isolates, the same method was applied with disks of 30 µg erythromycin, 10 µg clindamycin and 30 µg rokitamycin. The agar had been inoculated with a swab containing 0.5 McFarland bacterial suspensions [19].

2.3. DNA isolation and detection of macrolide resistance genes by polymerase chain reaction (PCR)

Strains were cultivated on blood agar (5% sheep blood). Chromosomal DNA was isolated using a standardised procedure (DNeasy Blood & Tissue Kit; QIAGEN, Hilden, Germany). The distinction between *mef*(A) and *mef*(E) was made using a multiplex PCR [20] with the following primers: *mef*AF, 5'-AATACAACAATTGGATT-3'; *mef*EF, 5'-AAGGAGTTGTGGTTCTGA-3'; and *mef*R, 5'-CCAATGATTTACACCGATT-3'. The PCR mixture for 25 µL contained 15 mM Tris–

HCl, 50 mM KCl (pH 8.0), 25 mM MgCl₂, 100 µM of each nucleotide, 0.15 mM of mefAF and mefEF primer and 0.25 mM of the mefR primer plus 1.5 U of AmpliTaq Gold® DNA Polymerase (Applied Biosystems, Darmstadt, Germany). Initial denaturation was at 94 °C for 12 min, followed by 30 cycles at denaturation at 94 °C for 1 min, annealing at 52 °C for 1 min and elongation at 72 °C for 1 min. Final elongation was performed at 72 °C for 10 min. To detect *erm*(B), the primers ermB up (5'-GAAAGGTACTCAACCAAATA-3') and ermB down (5'-AGTAACGGTACTTAAATTGTTTAC-3') were used with PCR conditions as described by Sutcliffe et al. [21]. For detection of the *erm*(A) gene in *S. pyogenes*, PCR with the following primers was performed: ermA up, 5'-TCTCCTTGCCGGTTATAA-3'; and ermA down, 5'-ATCAATTAAGACAGGTGCTGAAGC-3' [13]. A positive reference control strain was included in all PCR experiments. Products were run on a 1% agarose gel and were visualised by ethidium bromide staining.

2.4. Sequencing of macrolides resistance genes

DNA sequencing of four *S. pneumoniae* isolates, randomly selected from each of the resistance genotypes/phenotypes [*mef*(A), *mef*(E), *erm*(B) and *mef*(E)+*erm*(B)] was performed on an ABI 710 capillary sequencer (Applied Biosystems) according to the manufacturer's instructions using the PCR products and the same primers as used for the PCRs in order to confirm the PCR results. Resulting sequences were compared with the GenBank database using the program BLAST (<http://www.ncbi.nlm.nih.gov/BLAST>).

2.5. Typing

Strains were serotyped using the Neufeld Quellung reaction with type and factor sera provided by the Statens Serum Institut (Copenhagen, Denmark) [22]. All 437 macrolide-resistant *S. pneumoniae* isolates were available for serotyping. Multilocus sequence typing (MLST) of a randomly selected subgroup (166/437 isolates) of all pneumococcal isolates was performed as previously described [23]. Briefly, internal fragments of the *aroE*, *gdh*, *gki*, *recP*, *spi*, *xpt* and *ddl* genes were amplified by PCR from chromosomal DNA with the described primer pairs. A special allelic profile is provided by the alleles at each of the seven loci, and their sequence type (ST) is defined. Allelic profiles were compared with each other and with other isolates in the pneumococcal MLST database using software available at <http://www.mlst.net>. Clusters of related STs were grouped into clonal complexes (CCs) using the program eBURST (<http://www.mlst.net>).

3. Results

3.1. Bacterial strains

In total, 437 (21.4%) of 2045 *S. pneumoniae* and 29 (8.2%) of 352 *S. pyogenes* were found to be macrolide-resistant. Of the 437 pneumococci, 210 and 227 were isolated from individuals in the age groups 0 to <16 years and ≥ 16 years, respectively.

Streptococcus pneumoniae isolates were obtained from blood ($n = 304$), CSF ($n = 71$), nasopharyngeal swabs ($n = 31$), pleural fluid ($n = 6$), tracheal secretion or

sputum ($n = 12$), other normally sterile body sides ($n = 7$) and other sources such as urine, superficial wound swabs or bronchial secretion ($n = 6$). *Streptococcus pyogenes* isolates were from nasopharyngeal swabs ($n = 19$), blood ($n = 7$), punctures ($n = 2$) and bronchial secretion ($n = 1$).

3.2. Susceptibility testing

Among the 2045 pneumococcal isolates, the following rates of resistance were observed for all isolates/invasive isolates (from a normally sterile body side)/non-invasive isolates: clarithromycin, 21.4/20.6/30.6% (intermediate resistance to clarithromycin 2.6/7.7/20.4%); penicillin G, 3.6/0.0/0.0% (non-meningitis strains) (intermediate resistance to penicillin G for non-meningitis strains 4.8/1.6/10.2%), and 0.8/14.1% (meningitis strains from CSF) (no isolates with intermediate resistance); cefotaxime, 0.9/0.0/0.0% (non-meningitis) (intermediate resistance to cefotaxime 1.0/1.3/12.3%), and 0.3/0.6% (meningitis) (intermediate resistance to cefotaxime 0.0/1.3%); clindamycin, 7.5/6.4/20.1%; tetracycline, 10.2/8.8/27.2%; and levofloxacin, 0.6/0.0/0.0%. There were no isolates with intermediate resistance to clindamycin, tetracycline or levofloxacin.

Among the 352 *S. pyogenes* isolates, all isolates were susceptible to penicillin G, cefotaxime or levofloxacin. Moreover, 8.2% of the isolates were resistant to clarithromycin, 2.5% to clindamycin and 12.7% to tetracycline. Of the macrolide (clarithromycin)-resistant isolates of *S. pneumoniae* and *S. pyogenes* isolates, 36% and 21%, respectively, were found to be co-resistant to other antibiotics tested in this

study. *erm*(B)-positive isolates exhibiting the MLS_B phenotype had the highest rates of co-resistance to β -lactams (*S. pneumoniae*), clindamycin and tetracycline. In contrast, *mef*(A)- and *mef*(E)-positive isolates exhibiting the M-phenotype only rarely showed co-resistance (Table 1).

3.3. Genotypes, phenotypes, co-resistance and dual resistance mechanism

Of the 437 macrolide-resistant pneumococcal isolates, 252 (57.7%) carried the *mef*(A) gene, 49 (11.2%) carried the *mef*(E) gene, 118 (27%) carried the *erm*(B) gene and 18 (4.1%) carried *mef*(E)+*erm*(B) (see Table 2). All *mef*(A)- and *mef*(E)-positive *S. pneumoniae* isolates showed the M-phenotype. All *erm*(B)-positive isolates exhibited the MLS_B phenotype with clarithromycin MICs up to 32 μ g/mL. Clarithromycin MICs for M-phenotype strains (2–16 μ g/mL) were slightly lower compared with MLS_B phenotype strains. Serotypes for 430 macrolide-resistant isolates could be determined, whereas 7 isolates were non-typeable (NT). MLST was performed for a randomly selected subgroup (166/437 isolates) of isolates, comprising 31% ($n = 79$), 39% ($n = 46$), 55% ($n = 27$) and 78% ($n = 14$) of the isolates containing *mef*(A), *erm*(B), *mef*(E) and dual *mef*(E)+*erm*(B), respectively.

Of the 118 isolates harbouring *erm*(B), 87.2% and 94.1% were resistant to tetracycline and clindamycin, respectively; and the 18 isolates with *mef*(E)+*erm*(B) had co-resistance rates of 83.3% and 77.7% to tetracycline and clindamycin, respectively. Co-resistance rates to tetracycline and clindamycin were 24.5% and 2.0%, respectively, for the 49 *mef*(E)-positive isolates and 1.2% and 0.4% for the

252 *mef(A)*-positive isolates. In addition, 8 (42.1%) of 19 *erm(B)*-positive isolates from CSF, 1 (20.0%) of 5 *mef(E)*-positive isolates from CSF and 1 (33.3%) of 3 *mef(E)+erm(B)*-positive isolates from CSF were resistant to penicillin. Lastly, 9 (9.1%) of the 99 *erm(B)*-positive isolates from sources other than CSF (non-meningitis patients) and 1 (6.7%) of 15 *mef(E)+erm(B)*-positive isolates from sources other than CSF were intermediate resistant to penicillin, with an MIC of 4 µg/mL.

When applying the non-susceptibility breakpoint of ≥ 0.12 µg/mL for all *S. pneumoniae* isolates, isolates carrying *mef(E)*, *erm(B)* or *mef(E)+erm(B)* showed higher MICs for penicillin than *mef(A)*-carrying isolates. *mef(A)* isolates rarely have a penicillin MIC ≥ 0.12 µg/mL (0.8% of cases), whereas 20–25% of *mef(E)*-positive isolates (both CSF and non-CSF), 40–50% of *erm(B)*-positive isolates, and 33% (CSF) and 73% (non-CSF) of *mef(E)+erm(B)*-positive isolates have a penicillin MIC ≥ 0.12 µg/mL.

Interestingly, 18 (4.1%) of the macrolide-resistant *S. pneumoniae* isolates showed the dual resistance mechanism *mef(E)+erm(B)*, 9 of which were from non-invasive sites with an MLS_B phenotype and MICs for clarithromycin ranging from 16 µg/mL to 32 µg/mL. Various combinations of STs and serotypes were found for the 14 *mef(E)+erm(B)* isolates (Table 3) where MLST (ST), with CC and serotype information, was available: ST15, CC9, serotype 14 (*n* = 1); ST135, CC135, serotype 6B (*n* = 1); ST143, CC156, serotype 6B (*n* = 1), serotype 14 (*n* = 1); ST156, CC156, serotype 14 (*n* = 1); ST162, CC156, serotype 9V (*n* = 1); ST199, CC199, serotype 15B (*n* = 1); ST271, CC271, serotype 7F (*n* = 1), serotype 19F (*n* = 1); ST306,

CC306, serotype 1 ($n = 1$); SLV 306, CC306, serotype 1 ($n = 1$); ST310, CC393, serotype 19F ($n = 1$); ST2432, CC271, serotype 14 ($n = 1$); and ST3563, CC242, serotype 23F ($n = 1$).

Two of the 18 *mef*(E)+*erm*(B)-positive isolates (MLS_B phenotype) were co-resistant to penicillin G (one meningitis and one non-meningitis), 15 to clindamycin and 15 to tetracycline.

Four of the isolates with a dual resistance mechanism exhibited the M-phenotype, eight isolates had the constitutive MLS_B (cMLS_B) phenotype and six had the inducible MLS_B (iMLS_B) phenotype, as shown in repeated experiments (Table 2).

Of note, the majority of *mef*(A)-positive *S. pneumoniae* isolates (237/252; 94.0%) belonged to serotype 14. Other serotypes included 1 ($n = 1$), 6A ($n = 1$), 6B ($n = 1$), 7F ($n = 2$), 9N ($n = 1$), 9V ($n = 4$), 19F ($n = 2$) and 23F ($n = 3$). Of the 79 *mef*(A)-positive *S. pneumoniae* isolates with MLST data available, 69 were ST9 in combination with serotype 14, indicating clonal relatedness [*mef*(A)-positive England¹⁴⁻⁹]. Other combinations were ST15/serotype 14, ST138/serotype 14, ST191/serotype 14, ST350/serotype 14, DLV 9/serotype 14, SLV 1775/serotype 14, ST162/serotype 9V, ST176/serotype 6B, ST306/serotype 1 and ST2031/serotype 23F.

Among the *erm*(B)-positive isolates, serotypes 6B ($n = 32$), 19F ($n = 20$), 19A ($n = 19$) and 14 ($n = 16$) were the most predominant, in addition to 23F ($n = 7$), NT ($n =$

5), 9V ($n = 4$), 24F ($n = 4$), 6A ($n = 2$), 15A ($n = 2$), 1 ($n = 1$), 3 ($n = 1$), 4 ($n = 1$), 10A ($n = 1$), 15B ($n = 1$), 15C ($n = 1$) and 17F ($n = 1$). MLST data were available for 46 of the *erm*(B)-positive isolates, among which the most prevalent combination was ST135/serotype 6B ($n = 4$); other combinations were ST315/serotype 6B ($n = 3$) and ST193/serotype 19A ($n = 2$) (Table 3).

The 29 macrolide-resistant *S. pyogenes* showed the following distribution of resistance determinants: *mef*(A) ($n = 9$); *erm*(A) ($n = 4$); and *erm*(B) ($n = 10$). No resistance determinant could be detected in six isolates. There were no isolates harbouring a dual resistance mechanisms. Among the *S. pyogenes*, all *mef*(A)-positive isolates had an M-phenotype, whilst all *erm*(B) and *erm*(A)-positive isolates exhibited the MLS_B phenotype (Table 2).

3.4. Sequencing of macrolide resistance determinants

For each of the four possible combinations of resistance determinants [*mef*(A), *mef*(E), *erm*(B), and *mef*(E)+*erm*(B)] in *S. pneumoniae*, sequencing of the macrolide resistance gene(s) of one randomly selected isolate was performed. The sequences were obtained and compared with GenBank (<http://www.ncbi.nlm.nih.gov/BLAST>). The sequences were identical to those available in the database, thus confirming that the isolates indeed carried the two different macrolide resistance markers *mef*(A) and *mef*(E), as well as the combination of *mef*(E)+*erm*(B).

Primers for the multiplex PCR were designed based on a multiple sequence alignment of sequences belonging to both genes, revealing areas with nucleotide differences between the two genes and thus allow differentiation between *mef*(A) and *mef*(E). The primers are located within the genes.

4. Discussion

Macrolide resistance among streptococci has become an increasing problem worldwide [3,4]. In Germany, macrolide resistance rates in *S. pneumoniae* have further increased from 15.3% in 2000 [24] to 21.4% in 2005/2006 as analysed in the present study, and now in 2010 reach a level comparable with those reported from Mediterranean countries such as Spain (34.3%) and Italy (24.3%) [20]. In contrast, *S. pyogenes* isolates included in the present study showed a macrolide resistance rate of 8.2%, which is significantly lower than the resistance rates reported from Spain (34%) and Italy (35%) [25,26] and even somewhat lower than in earlier reports from Germany, with a 14% resistance level reported among children up to 16 years of age with community-acquired infections in 2004 [17], or a rate of 19.1% (adults) and 11.8% (children) reported from Bavaria in 2003 [27].

In Germany, macrolide resistance in *S. pneumoniae* is mainly due to *mef* genes. In all previous studies performed in Germany, no distinction between *mef*(A) and *mef*(E) determinants was made [28]. In some Mediterranean countries and Canada, *mef*(E) appears to be the most common efflux gene variant [20,29]. In Germany, *mef*(A) is the most common macrolide resistance gene.

Macrolide-resistant *S. pneumoniae* strains were increasingly found to be co-resistant to other compounds, such as tetracycline (31%), clindamycin (29%) and penicillin (2% meningitis/2% non-meningitis). In Germany, multidrug-resistant (MDR) isolates predominantly exhibit the MLS_B phenotype and carry *erm*(B). Multiple antibiotic resistance is widespread in Europe and an increase in MDR *S. pneumoniae* and *S. pyogenes* strains has also been observed in other European countries such as Spain [4,20,30].

Streptococcus pneumoniae isolates carrying *mef*(E), *erm*(B) or *mef*(E)+*erm*(B) showed higher MICs for penicillin than *mef*(A)-carrying isolates. This probably reflects the greater clonal diversity among the *mef*(E)- and *erm*(B)-positive isolates. Obviously, the chance of acquiring macrolide resistance elements in low-affinity penicillin-binding protein strains is then higher.

Of note, this is the first study to report a relatively high level of isolates with the dual resistance mechanism [both *erm*(B) and *mef*(A), subclass *mef*(E) gene] from Germany. Similar isolates were first described in South Africa in 2001 [31] and in the last decade have been increasingly reported from the USA, Canada, South Korea, China, South Africa, Japan, Mexico and Hungary [32]. In the present study, which differentiated between *mef*(A) subclasses *mef*(A) and *mef*(E), only isolates carrying the *mef*(E) gene, but not the *mef*(A) gene, with *erm*(B) were observed, underscoring the different genetic background of *mef*(E) and *mef*(A). Moreover, two isolates belonged to CC271, which is the most predominant clone in the USA, where >90%

of isolates with the dual resistance mechanism were clonally related to the MDR international clone Taiwan^{19F}-14 (CC271) [32]. This cross-resistance could be related to the presence of conjugative transposons such as Tn2010 described for these isolates [33]. Apart from the *erm*(B) gene, Tn2010 also harbours a tetracycline resistance gene. The MEGA element, which encodes the *mef*(E) gene, can be inserted in Tn2010 but also in various other insertion sites on the chromosome [7,11,20]. Of note, four of the isolates with the dual resistance mechanism exhibited the M-phenotype and remained sensitive to the lincosamide clindamycin in repeated experiments, indicating that the *erm*(B) gene in these isolates has no functional activity.

In the past there has been discussion regarding the nomenclature of the *mef* genes. The first *mef* gene described in *S. pyogenes* was *mef*(A). Later, a homologous *mef* gene was found in *S. pneumoniae* and was named *mef*(E) [34]. After both genes were found in both species and their sequences were found to be 90% identical, Roberts et al. [14] suggested to rename all the genes *mef*(A) and to refer to the two variants as subclasses *mef*(A) and *mef*(E). However, since the *mef*(A) subclasses *mef*(A) and *mef*(E) are carried by different genetic elements, which are inserted at different sites into the chromosome, they showed different clonal dispersion. Owing to these differences, it was suggested to maintain the distinction between the two genes [34]. The findings of the present paper clearly support the view that both genes have a different genetic background as documented by their association with different CCs and further support the view of Klaassen et al. [34].

In conclusion, macrolide resistance in *S. pneumoniae*, but not *S. pyogenes*, is further increasing in Germany. In pneumococci, macrolide resistance is mainly caused by the spread of a single clone (England¹⁴⁻⁹) carrying the *mef(A)* gene. For the first time, isolates with a dual macrolide resistance mechanism were observed in Germany. Moreover, this study underscores that a differentiation between *mef(A)* and *mef(E)* gene variants provides a better insight into the spread of macrolide resistance among *S. pneumoniae* and *S. pyogenes* in different geographical locations.

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Competing interests

MvdL has received research grants from Wyeth/Pfizer and GSK and acts as a consultant for Wyeth/Pfizer, Sanofi-Aventis and GSK. RRR has been a Novartis Vaccines and Diagnostics employee since 2010 and was a Wyeth Vaccines employee from 2007 to 2010. CB declares no competing interests.

Ethical approval

Not required.

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Table 1

Minimal inhibitory concentrations (MICs) and co-resistance among macrolide-resistant isolates of *Streptococcus pneumoniae* (S.p.) in Germany, 2005–2006

Resistance	Clarithromycin				Penicillin			Clindamycin			Tetracycline		
genotype (no. of isolates)	MIC _{50/90} (µg/mL)	MIC range (µg/mL)	% NS of all 2045 S.p.	% of all CLA ^R S.p. (n = 437)	MIC _{50/90}	MIC range	% NS (I+R) of 437 CLA ^R S.p. (meningitis/non- meningitis) ^a	MIC _{50/90}	MIC range	% NS of 437 CLA ^R S.p. ^a	MIC _{50/90}	MIC range	% NS of 437 CLA ^R S.p. ^a
mef(A) (252)	8/16	2–16	12.3	57.7	0.015/0.015	0.015– 0.06	0/0	0.12/0.12	0.12– 0.12	0.4	0.25/0.5	0.06– 32	1.2
mef(E) (49)	8/16	2–16	2.4	11.2	0.015/0.25	0.015– 2	20.0/0	0.12/0.12	0.12– 32	2.0	0.5/16	0.25– 16	24.5
mef(E)+erm(B) (18)	32/32	2–32	0.9	4.1	0.25/2	0.015– 4	33.3/6.7	16/32	0.12– 32	77.7	16/32	0.12– -64	83.3
erm(B) (118)	32/32	2–32	5.8	27.0	0.06/2	0.015– 4	42.1/9.1	32/32	0.5– 32	94.1	32/32	0.25– 32	87.2

MIC_{50/90}, MICs for 50% and 90% of the isolates, respectively; NS, non-susceptibility; CLA^R, clarithromycin-resistant; intermediate; R, resistant.

^a The following breakpoints (µg/mL) were used (susceptible/intermediate/resistant): penicillin (parenteral), ≤0.06, – and ≥0.12 (meningitis)/≤2, 4 and ≥8 (non-meningitis); clindamycin, ≤0.25, 0.5 and ≥1; tetracycline, ≤2, 4 and ≥8; and levofloxacin, ≤2, 4 and ≥8 (all macrolide-resistant isolates were susceptible to levofloxacin).

Table 2

Phenotypes and genotypes of 437 macrolide-resistant *Streptococcus pneumoniae* and 29 macrolide-resistant *Streptococcus pyogenes* in Germany, 2005–2006

<i>S. pneumoniae</i> (No. of isolates)	Phenotype	No. of isolates with genotype:			
		<i>mef</i> (A)	<i>mef</i> (E)	<i>mef</i> (E)+ <i>erm</i> (B)	<i>erm</i> (B)
Adults, invasive disease (207)	M	125	21	0	0
	cMLS _B	0	0	1	47
	iMLS _B	0	0	1	12
	Total	125	21	2	59
Children, invasive disease (181)	M	122	19	2	0
	cMLS _B	0	0	4	28
	iMLS _B	0	0	1	5
	Total	122	19	7	33
Adults, non-invasive disease (20)	M	2	5	1	0
	cMLS _B	0	0	1	5
	iMLS _B	0	0	2	4
	Total	2	5	4	9
Children, non-invasive disease (29)	M	3	4	1	0
	cMLS _B	0	0	2	11
	iMLS _B	0	0	2	6
	Total	3	4	5	17
<i>S. pyogenes</i> (No. of isolates)		<i>mef</i> (A)	<i>mef</i> (E)	<i>erm</i> (B)	<i>erm</i> (A)
Adults (19) ^a	M	3	0	0	0
	cMLS _B	0	0	7	0
	iMLS _B	0	0	2	2
	None	0	0	1	0
	Total	3	0	10	2
Children (10) ^a	M	6	0	0	0
	cMLS _B	0	0	0	1

iMLS _B	0	0	0	1
None	0	0	0	0
Total	6	0	0	2

cMLS_B, constitutive macrolide–lincosamide–streptogramin B (MLS_B) phenotype;

iMLS_B, inducible MLS_B phenotype.

^a No resistance gene could be detected in four *S. pyogenes* isolates from adults and two isolates from children.

Table 3

Clonal distribution and macrolide resistance markers among 166 randomly selected *Streptococcus pneumoniae* isolates in Germany, 2005–2006

Sequence type	Clonal complex (CC)	N	Gene	Serotype
9	CC9	70	<i>mef</i> (A) (<i>n</i> = 69) <i>erm</i> (B) (<i>n</i> = 1)	14 (<i>n</i> = 69) 14 (<i>n</i> = 1)
242	CC242	8	<i>mef</i> (E) (<i>n</i> = 8)	23F (<i>n</i> = 7), 19F (<i>n</i> = 1)
162	CC156	8	<i>mef</i> (E) (<i>n</i> = 6) <i>mef</i> (E)+ <i>erm</i> (B) (<i>n</i> = 1) <i>mef</i> (A) (<i>n</i> = 1)	9V (<i>n</i> = 5), nt (<i>n</i> = 1) 9V (<i>n</i> = 1) 9V (<i>n</i> = 1)
135	CC135	5	<i>mef</i> (E)+ <i>erm</i> (B) (<i>n</i> = 1) <i>erm</i> (B) (<i>n</i> = 4)	6B (<i>n</i> = 1) 6B (<i>n</i> = 3), nt (<i>n</i> = 1)
15	CC9	5	<i>mef</i> (A) (<i>n</i> = 1) <i>mef</i> (E) (<i>n</i> = 1) <i>mef</i> (E)+ <i>erm</i> (B) (<i>n</i> = 1) <i>erm</i> (B) (<i>n</i> = 2)	14 (<i>n</i> = 1) 14 (<i>n</i> = 1) 14 (<i>n</i> = 1) 14 (<i>n</i> = 2)
143	CC156	4	<i>mef</i> (E)+ <i>erm</i> (B) (<i>n</i> = 2) <i>erm</i> (B) (<i>n</i> = 2)	6B (<i>n</i> = 1), 14 (<i>n</i> = 1) 14 (<i>n</i> = 2)
191	CC191	4	<i>mef</i> (A) (<i>n</i> = 1) <i>mef</i> (E) (<i>n</i> = 3)	14 (<i>n</i> = 1) 7F (<i>n</i> = 3)
315	CC315	4	<i>erm</i> (B) (<i>n</i> = 4)	6B (<i>n</i> = 3), 19A (<i>n</i> = 1)
138	CC176	3	<i>erm</i> (B) (<i>n</i> = 2) <i>mef</i> (A) (<i>n</i> = 1)	6B (<i>n</i> = 2) 14 (<i>n</i> = 1)

179	CC177	3	<i>erm</i> (B) (<i>n</i> = 3)	19F (<i>n</i> = 3)
193	CC193	3	<i>erm</i> (B) (<i>n</i> = 3)	19A (<i>n</i> = 2), 15C (<i>n</i> = 1)
473	CC473	3	<i>mef</i> (E) (<i>n</i> = 1) <i>erm</i> (B) (<i>n</i> = 2)	6A (<i>n</i> = 1) 6B (<i>n</i> = 1), 23F (<i>n</i> = 1)
1848	CC207	3	<i>erm</i> (B) (<i>n</i> = 3)	19A (<i>n</i> = 3)
81	CC81	2	<i>mef</i> (E) (<i>n</i> = 1) <i>erm</i> (B) (<i>n</i> = 1)	19F (<i>n</i> = 1) 19F (<i>n</i> = 1)
156	CC156	2	<i>mef</i> (E) (<i>n</i> = 1) <i>mef</i> (E)+ <i>erm</i> (B) (<i>n</i> = 1)	14 (<i>n</i> = 1) 14 (<i>n</i> = 1)
176	CC176	2	<i>mef</i> (A) (<i>n</i> = 1) <i>erm</i> (B) (<i>n</i> = 1)	6B (<i>n</i> = 1) 6B (<i>n</i> = 1)
199	CC199	2	<i>mef</i> (E) (<i>n</i> = 1) <i>mef</i> (E)+ <i>erm</i> (B) (<i>n</i> = 1)	19A (<i>n</i> = 1) 15B (<i>n</i> = 1)
271	CC271	2	<i>mef</i> (E)+ <i>erm</i> (B) (<i>n</i> = 2)	7F (<i>n</i> = 1), 19F (<i>n</i> = 1)
273	CC90	2	<i>erm</i> (B) (<i>n</i> = 2)	6A (<i>n</i> = 1), 6B (<i>n</i> = 1)
306	CC306	2	<i>mef</i> (A) (<i>n</i> = 1) <i>mef</i> (E)+ <i>erm</i> (B) (<i>n</i> = 1)	1 (<i>n</i> = 1) 1 (<i>n</i> = 1)
350	CC304	2	<i>mef</i> (A) (<i>n</i> = 1) <i>mef</i> (E) (<i>n</i> = 1)	14 (<i>n</i> = 1) 14 (<i>n</i> = 1)
507	CC439	2	<i>erm</i> (B) (<i>n</i> = 2)	23F (<i>n</i> = 2)
63	CC63	1	<i>erm</i> (B) (<i>n</i> = 1)	15A (<i>n</i> = 1)
158	CC156	1	<i>erm</i> (B) (<i>n</i> = 1)	14 (<i>n</i> = 1)
180	CC180	1	<i>erm</i> (B) (<i>n</i> = 1)	3 (<i>n</i> = 1)
230	CC230	1	<i>erm</i> (B) (<i>n</i> = 1)	19A (<i>n</i> = 1)

276	CC230	1	<i>erm</i> (B) (<i>n</i> = 1)	19A (<i>n</i> = 1)
310	CC393	1	<i>mef</i> (E)+ <i>erm</i> (B) (<i>n</i> = 1)	19F (<i>n</i> = 1)
319	CC230	1	<i>erm</i> (B) (<i>n</i> = 1)	19F (<i>n</i> = 1)
320	CC271	1	<i>mef</i> (E) (<i>n</i> = 1)	19A (<i>n</i> = 1)
416	CC199	1	<i>erm</i> (B) (<i>n</i> = 1)	19A (<i>n</i> = 1)
1171	CC177	1	<i>erm</i> (B) (<i>n</i> = 1)	19F (<i>n</i> = 1)
1292	CC1397	1	<i>mef</i> (E) (<i>n</i> = 1)	6A (<i>n</i> = 1)
1348	CC135	1	<i>erm</i> (B) (<i>n</i> = 1)	6B (<i>n</i> = 1)
1518	CC176	1	<i>mef</i> (E) (<i>n</i> = 1)	6B (<i>n</i> = 1)
1621	CC63	1	<i>erm</i> (B) (<i>n</i> = 1)	19A (<i>n</i> = 1)
1984	CC156	1	<i>erm</i> (B) (<i>n</i> = 1)	9V (<i>n</i> = 1)
2031	CC176	1	<i>mef</i> (A) (<i>n</i> = 1)	23F (<i>n</i> = 1)
2355	CC230	1	<i>erm</i> (B) (<i>n</i> = 1)	17F (<i>n</i> = 1)
2432	CC271	1	<i>mef</i> (E)+ <i>erm</i> (B) (<i>n</i> = 1)	14 (<i>n</i> = 1)
2550	CC177	1	<i>mef</i> (E) (<i>n</i> = 1)	19F (<i>n</i> = 1)
2652	CC9	1	<i>erm</i> (B) (<i>n</i> = 1)	14 (<i>n</i> = 1)
3563	CC242	1	<i>mef</i> (E)+ <i>erm</i> (B) (<i>n</i> = 1)	23F (<i>n</i> = 1)
SLV 124	CC124	1	<i>erm</i> (B) (<i>n</i> = 1)	14 (<i>n</i> = 1)
SLV 306	CC306	1	<i>mef</i> (E)+ <i>erm</i> (B) (<i>n</i> = 1)	1 (<i>n</i> = 1)
SLV 1775	CC9	1	<i>mef</i> (A) (<i>n</i> = 1)	14 (<i>n</i> = 1)
DLV 9	CC9	1	<i>mef</i> (A) (<i>n</i> = 1)	14 (<i>n</i> = 1)

nt, non-typeable.