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ARTICLE

Penicillin susceptibility breakpoints for *Streptococcus pneumoniae* and their effect on susceptibility categorisation in Germany (1997–2013)

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Abstract Continuous nationwide surveillance of invasive pneumococcal disease (IPD) was conducted in Germany. From July 1, 1997, to June 30, 2013, data on penicillin susceptibility were available for 20,437 isolates. 2,790 of these isolates (13.7 %) originate from patients with meningitis and 17,647 isolates (86.3 %) are from non-meningitis cases. A slight decline in isolates susceptible at 0.06 and 0.12 μg/ml can be noticed over the years. Overall, 89.1 % of the isolates had minimum inhibitory concentrations (MICs) of ≤0.015 μg/ml. In 2012/2013, the first three isolates of *Streptococcus pneumoniae* with MICs of 8 μg/ml were found. The application of different guidelines with other MIC breakpoints for the interpretation of penicillin resistance leads to differences in susceptibility categorisation. According to the pre-2008 Clinical and Laboratory Standards Institute (CLSI) interpretive criteria, 5.3 % of isolates overall were intermediate and 1.4 % were resistant to penicillin. Application of the 2008–2014 CLSI interpretive criteria resulted in 7.6 % resistance among meningitis cases and 0.5 % intermediate resistance in non-meningitis cases. Referring to the 2009–2014 European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints, 7.6 % of the isolates in the meningitis group were resistant to penicillin. In the non-meningitis group, 6.1 % of the isolates were intermediate and 0.5 % were resistant. These differences should be kept in mind when surveillance studies on pneumococcal penicillin resistance are compared.

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Introduction

Streptococcus pneumoniae is a leading pathogen in bacterial pneumonia, sepsis and meningitis worldwide [1]. Penicillin still is the preferred antimicrobial agent for susceptible *S. pneumoniae* infections.

The Clinical and Laboratory Standards Institute (CLSI) breakpoints used for the interpretation of minimum inhibitory concentrations (MICs) till 2007 were based on concentrations that could be reached in cerebrospinal fluid (CSF) to assure the successful treatment of meningitis cases. In 2008, new breakpoints were published by the CLSI as a result of accumulated evidence for the successful treatment of non-meningitis pneumococcal infections, even when the pre-2008 breakpoints suggest a reduced susceptibility for these isolates in vitro. According to these interpretive criteria, different breakpoints for the parenteral use of penicillin concerning meningitis and non-meningitis cases have to be applied: for meningitis cases, the intermediate category was omitted and higher breakpoints for non-meningitis cases were defined. For oral application, the old interpretive criteria were maintained (Table 1 in the Appendix) [2–6].

Interpretive criteria of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) were published in 2009 for the first time. These guidelines also specify different breakpoints for meningitis and non-meningitis cases. However, while the meningitis breakpoints are identical to the 2008–2014 CLSI values, the breakpoints in non-meningitis cases are considerably lower (Table 1 in the Appendix) [7, 8].

The aim of this study was, in addition to our previously published data on the effects of the 2008 CLSI breakpoints on

susceptibility categorisation [9], to analyse trends in penicillin MIC development among all isolates of *S. pneumoniae* with invasive pneumococcal disease (IPD) that were sent to the German National Reference Center for Streptococci (NRCS) between July 1997 and June 2013, and to evaluate differences in susceptibility categorisation when applying the interpretive criteria used by the CLSI before 2008, by the CLSI after 2008 and by the EUCAST, respectively.

Materials and methods

Study design

The NRCS has conducted surveillance for IPD in Germany since 1992. In the current study, a population- and laboratory-based approach was used to collect data about IPD in Germany. The isolates were sent to the NRCS by diagnostic microbiological laboratories throughout Germany on a voluntary basis. *S. pneumoniae* samples isolated from blood, CSF or other normally sterile body sites from July 1, 1997, to June 30, 2013, were included in the study.

Microbiological investigations

Isolates were identified by standard procedures, including bile solubility and optochin sensitivity. MIC testing was performed using the broth microdilution method as recommended by the CLSI [3, 4]. With regard to susceptibility categorisation according to the CLSI interpretive criteria in 2008, only the breakpoints set for parenteral use of the corresponding antibiotics were applied. Since the MIC testing of the isolates strictly referred to the CLSI recommendations, the EUCAST breakpoints were used for reasons of comparison only. *Streptococcus pneumoniae* ATCC 49619 was used as a control strain.

Results

A total of 20,549 isolates from IPD patients were collected during the study period. The numbers of cases for each epidemiological year (July 1 to June 30) vary between 377 and 2,533 (mean: 1,284.3).

Data on penicillin susceptibility were available for 20,437 isolates, with 2,790 (13.7 %) originating from patients with meningitis and 17,647 (86.3 %) from non-meningitis cases.

The percentage of all invasive isolates susceptible at 0.06, 0.12, 0.25, 0.5, 1, 2 and 4 μg/ml is shown in Fig. 1. A slight decline in isolates susceptible at 0.06 and 0.12 μg/ml can be noticed over the years. In 2012/2013, the first three isolates of *S. pneumoniae* with MICs of 8 μg/ml during the study period were found [serotypes: 19A (2), 14 (1)]. No pronounced changes were observed for penicillin susceptibility at 0.25, 0.5, 1 and 2 μg/ml.

From the 20,437 isolates with data on penicillin susceptibility, 89.1 % had MICs of ≤0.015 μg/ml. The distribution of all MICs is illustrated in Fig. 2.

According to the pre-2008 CLSI interpretive criteria, 5.3 % of isolates overall were intermediate and 1.4 % were resistant to penicillin. The development of resistance rates, when classified according to the pre-2008 CLSI breakpoints (which are identical to the oral CLSI 2008–2014 breakpoints) over the years, is shown in Fig. 3. When the isolates are classified into meningitis and non-meningitis cases, which is not indicated by the former interpretive criteria, a slightly higher rate of non-susceptibility is observed among the meningitis cases (6.5 % intermediate, 1.1 % resistant) than in the non-meningitis group (5.2 % intermediate, 1.4 % resistant) (Fig. 4).

When the 2008–2014 CLSI interpretive criteria are applied, higher resistance rates are detected in the meningitis group (7.6 % resistant) than among the non-meningitis cases (0.0 % resistant, 0.5 % intermediate). When summing up resistance rates from the meningitis and the non-meningitis-group, overall, 0.4 % of all isolates are intermediate and 1.1 % are resistant to penicillin (Table 2 in the Appendix, Fig. 4).

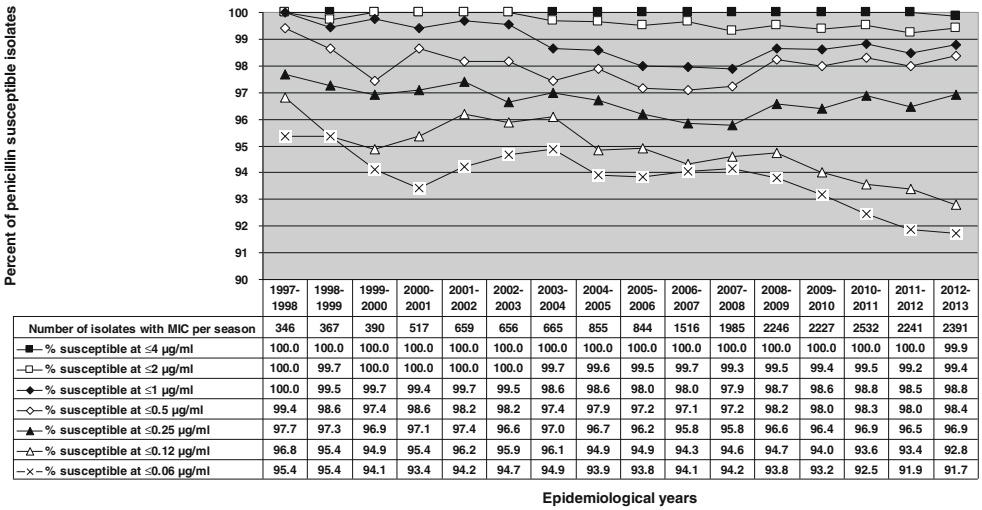
According to the 2009–2014 EUCAST breakpoints, 7.6 % of the isolates in the meningitis group were resistant to penicillin. In the non-meningitis group, 6.1 % of the isolates were intermediate and 0.5 % were resistant. These rates are higher than the non-susceptibility rates according to the 2008–2014 CLSI interpretive criteria. Summing up resistance rates from both groups, 5.2 % of all isolates overall are intermediate and 1.5 % are resistant to penicillin (Table 2 in the Appendix, Fig. 4).

Discussion

In this paper, we present the results of 16 years of surveillance concerning penicillin susceptibility of IPD in Germany.

In the last decade(s), the prevalence of antibiotic-resistant *S. pneumoniae* strains has been described to increase worldwide [10, 11]. In Europe, high resistance rates for penicillin have been reported from France, Spain and Eastern European countries, while Germany and the Northern European countries were affected to a lesser extent [12]. However, there have also been reports on declining resistance rates, especially in the past few years [13–17].

The interpretation and comparison of resistance data published from different countries is increasingly complex, because of the change in the interpretive criteria by the CLSI in 2008 [3] and the differences with the EUCAST criteria as published since 2009 [7]. Ideally, changing breakpoints should lead to a retrospective re-analysis of historical data to



	1997-1998	1998-1999	1999-2000	2000-2001	2001-2002	2002-2003	2003-2004	2004-2005	2005-2006	2006-2007	2007-2008	2008-2009	2009-2010	2010-2011	2011-2012	2012-2013
Number of isolates with MIC per season	346	367	390	517	659	656	665	855	844	1516	1985	2246	2227	2532	2241	2391
% susceptible at ≤4 µg/ml	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.9
% susceptible at ≤2 µg/ml	100.0	99.7	100.0	100.0	100.0	100.0	99.7	99.6	99.5	99.7	99.3	99.5	99.4	99.5	99.2	99.4
% susceptible at ≤1 µg/ml	100.0	99.5	99.7	99.4	99.7	99.5	98.6	98.6	98.0	98.0	97.9	98.7	98.6	98.8	98.5	98.8
% susceptible at ≤0.5 µg/ml	99.4	98.6	97.4	98.6	98.2	98.2	97.4	97.9	97.2	97.1	97.2	98.2	98.0	98.3	98.0	98.4
% susceptible at ≤0.25 µg/ml	97.7	97.3	96.9	97.1	97.4	96.6	97.0	96.7	96.2	95.8	95.8	96.6	96.4	96.9	96.5	96.9
% susceptible at ≤0.12 µg/ml	96.8	95.4	94.9	95.4	96.2	95.9	96.1	94.9	94.9	94.3	94.6	94.7	94.0	93.6	93.4	92.8
% susceptible at ≤0.06 µg/ml	95.4	95.4	94.1	93.4	94.2	94.7	94.9	93.9	93.8	94.1	94.2	93.8	93.2	92.5	91.9	91.7

Fig. 1 Percentage of isolates susceptible at 0.06, 0.12, 0.25, 0.5, 1, 2 and 4 µg/ml of all invasive isolates (1997/1998–2012/2013, *n*=20,437)

minimise misconstruing the resistance trends [17]. This should be kept in mind when interpreting surveillance studies on pneumococcal penicillin resistance.

All isolates associated with meningitis categorised as intermediate before 2008 by the CLSI interpretive criteria are now classified as resistant under the 2008–2014 CLSI interpretive criteria, leading to higher resistance rates among meningitis cases. In contrast, considerably lower resistance rates result for non-meningitis cases, due to the increased MIC value for the parenteral treatment of non-meningitis cases [2, 3, 5, 15, 17–20]. The increased MIC breakpoint reflects the fact that there are few cases of treatment failure of non-meningeal infections with high-dosage parenteral penicillin G, which still remains highly effective for many pneumococcal diseases [21], and is expected to reflect the clinical effectiveness of penicillin more accurately [15] and reduce the inconsistency between susceptibility results and therapeutic outcomes [19]. Since more non-meningitis isolates than meningitis isolates are normally sent to the reporting laboratories, the application of the 2008–2014 interpretive criteria results, presumably, in a lower overall rate of penicillin-resistant pneumococcal isolates, due to the numerical predominance of non-meningitis cases [9].

The influence of the change in breakpoints may lead to misinterpretation of resistance trends. For example, in their analysis of Belgian data from 2003 to 2010, Goossens et al. [17] described a significant decreasing trend in the proportion of penicillin non-susceptible *S. pneumoniae* (9.4 % to <1 %) for both blood and CSF isolates. However, 75 % of this decrease was explained by the change in the CLSI breakpoints. There was no significant decreasing trend when

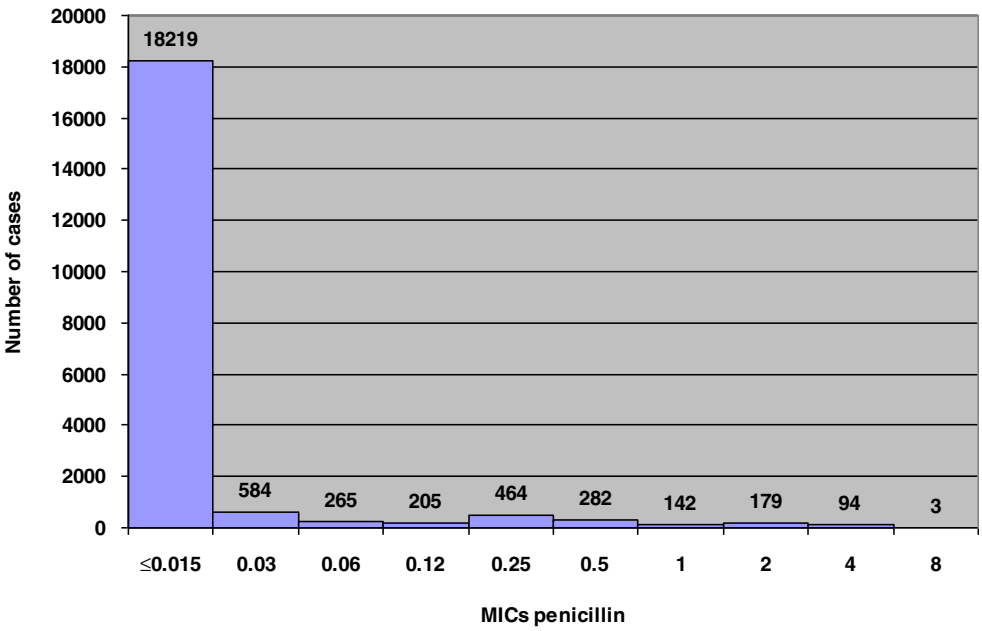


Fig. 2 MICs of all invasive isolates (1997/1998–2012/2013, *n*=20,437)

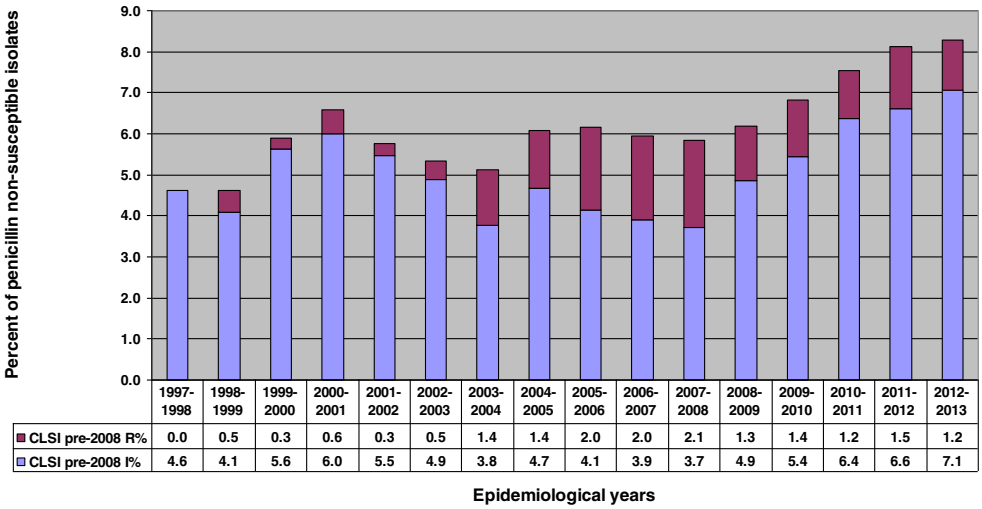


Fig. 3 Resistance rates of *S. pneumoniae* isolates according to the pre-2008 CLSI interpretive criteria, in percent

the pre-2008 CLSI breakpoints or the 2008–2014 CLSI breakpoints were used alone [17].

The EUCAST breakpoints, first published in 2009, correspond to the 2008–2014 CLSI interpretive criteria for meningitis isolates, while for non-meningitis isolates, the breakpoints are closer to the pre-2008 CLSI criteria. Accordingly, the rate of intermediate or resistant non-meningitis isolates was considerably higher with the EUCAST breakpoints in our study than when the 2008–2014 CLSI breakpoints were used. This is in accordance with results from Belgium, where the proportion of penicillin non-susceptible isolates was 7–13 times higher with the EUCAST breakpoints than with the new CLSI breakpoints [17].

The slight decline in isolates susceptible at 0.06 and 0.12 μg/ml over the years in our study is suggestive of a slow increase of pneumococcal penicillin resistance (MIC creep). The phenomenon has been described among 97,843 US isolates from the Surveillance Network® database for the period 1996–2008 recently as well [22]. While isolates from Taiwan showed an alarming increase in the number of borderline penicillin MICs (1–2 mg/l) during 2000–2007 (together with a decreasing trend of 4 mg/l MICs) [15], we could not find a comparable increase in isolates susceptible at 0.25, 0.5, 1 and 2 μg/ml in our study. However, there was a slight increase of *S. pneumoniae* isolates with 4 μg/ml MICs during the period of our study and, furthermore, in 2012/2013, the first three isolates with MICs of 8 μg/ml were found.

Among the factors that might have influenced the MIC development most profoundly, independent from the breakpoint interpretative criteria described here in detail, the general recommendation of pneumococcal conjugate vaccination for children<2 years old in Germany at the end of July 2006 has to be mentioned. In Germany, high rates of serotype-specific resistance among the more frequent serotypes were observed for the serotypes 19A, 9V, 6B, 19F, 23F and 14 between 1992 and 2008 [23]. Five of these serotypes were included in the 7-valent pneumococcal conjugate vaccine and serotype 19A is included in the 13-valent pneumococcal

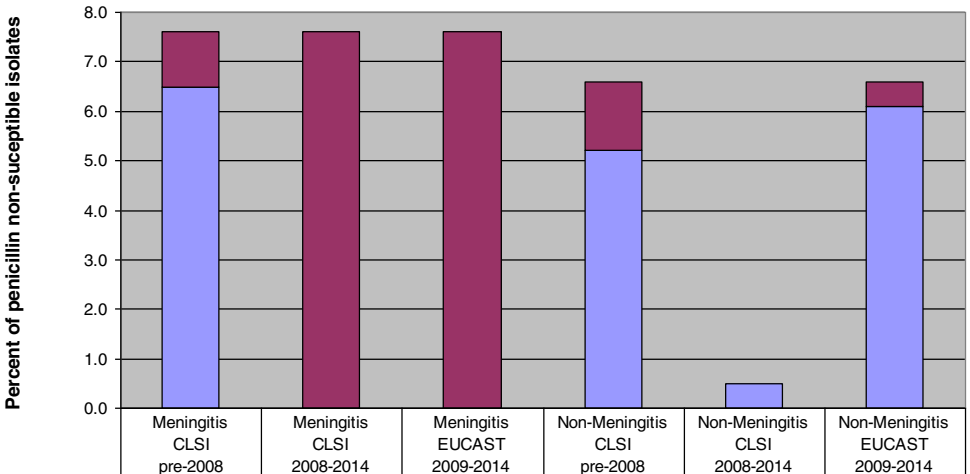


Fig. 4 Resistance rates of *S. pneumoniae* isolates among meningitis and non-meningitis cases according to the pre-2008 CLSI, CLSI 2008–2014 and EUCAST 2009–2014 breakpoints, in percent

conjugate vaccine. With the decrease of serotypes included in the pneumococcal conjugate vaccine, the shift in the pneumococcal-resistant population affects not only vaccinated children but also unvaccinated children and adults, as a result of a herd effect [13, 24, 25]. Since pneumococcal conjugate vaccines have been described to lead to a reduction in antibiotic use [26], they might also contribute to a lower antimicrobial selection pressure driving changes in the frequency of antibiotic resistance [13].

However, the normal temporal [10, 27, 28] and regional [12, 29, 30] variations of serotype distribution also have to be taken into consideration. To observe future developments in pneumococcal susceptibility to penicillin, continued surveillance is of great importance.

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Conflict of interest Ralf R. Reinert is an employee of Pfizer Vaccines. The university of Paul M. Tulkens has received research grants and speaker's honoraria from Cempra Pharmaceuticals, Bayer AG, GSK and AstraZeneca. Mark van der Linden has received research grants and speaker's honoraria from Pfizer, Sanofi Pasteur MSD, MSD and GSK.

Appendix

Table 1 CLSI and EUCAST breakpoints for *Streptococcus pneumoniae*

CLSI pre-2008 penicillin interpretations for *Streptococcus pneumoniae*

	Susceptible (μg/ml)	Intermediate (μg/ml)	Resistant (μg/ml)
	≤0.06	0.12–1[a]	≥2

[a]High doses of intravenous penicillin (e.g. at least 2 million units every 4 h in adults with normal renal function) are effective in treating pneumococcal pneumonia due to strains in the intermediate category.

CLSI 2008–2014 penicillin interpretations for *Streptococcus pneumoniae*

	Susceptible (μg/ml)	Intermediate (μg/ml)	Resistant (μg/ml)
Parenteral, non-meningitis	≤2	4	≥8
Parenteral, meningitis	≤0.06	–	≥0.12
Oral	≤0.06	0.12–1	≥2

EUCAST 2009–2014 penicillin interpretations for *Streptococcus pneumoniae*

	Susceptible (mg/l)	Resistant (mg/l)
Infections other than meningitis[b]	≤0.06	>2
Meningitis	≤0.06	>0.06

[b]In pneumonia, when a dose of 1.2 g × 4 is used, isolates with MIC ≤0.5 mg/L should be regarded as susceptible. In pneumonia, when a dose of 2.4 g × 4 or 1.2 g × 6 is used, isolates with MIC ≤1 mg/L should be regarded as susceptible. In pneumonia, when a dose of 2.4 g × 6 is used, isolates with MIC ≤2 mg/L should be regarded as susceptible.

Table 2 Resistance rates of *S. pneumoniae* isolates among meningitis and non-meningitis cases according to the CLSI 2008–2014 (parenterally administered antibiotics) and EUCAST 2009–2014 interpretive criteria per epidemiological year, in percent

Epidemiological year	Meningitis cases			Non-meningitis cases		
CLSI 2008–2014 interpretive criteria						
	Susceptible (%)	Intermediate (%)	Resistant (%)	Susceptible (%)	Intermediate (%)	Resistant (%)
2008–2009	93.7	0.0	6.3	99.5	0.5	0.0
2009–2010	93.4	0.0	6.6	99.3	0.7	0.0
2010–2011	91.5	0.0	8.5	99.5	0.5	0.0
2011–2012	88.7	0.0	11.3	99.3	0.7	0.0
2012–2013	88.1	0.0	11.9	99.4	0.5	0.1
EUCAST 2009–2014 interpretive criteria						
	Susceptible (%)	Intermediate (%)	Resistant (%)	Susceptible (%)	Intermediate (%)	Resistant (%)
2009–2010	93.4	0.0	6.6	93.1	6.2	0.7
2010–2011	91.5	0.0	8.5	92.6	7.0	0.5
2011–2012	88.7	0.0	11.3	92.2	7.1	0.7
2012–2013	88.1	0.0	11.9	92.1	7.3	0.6

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