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Detection of internationally spread multiresistant *Streptococcus pneumoniae* clones in Germany using Multilocus Sequence Typing

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ABSTRACT

Objectives: Multiresistant S. pneumoniae (SP) strains have become a global concern. In global spread of multiresistant SP only a few clonal complexes are widely distributed. By international comparison resistance in SP is low in Germany. The aim of our study was to investigate whether in Germany, despite low resistance rates, internationally spread multiresistant SP clones occur in isolates recovered from outpatients with RTIs.

Methods: We performed two multicenter studies in Germany in the winter of 2000/2001. MICs were determined using the broth microdilution procedure following NCCLS. Among others erythromycin (ERY), penicillin (PEN), tetracycline (TET) and co-trimoxazole (SXT) were tested. Resistance to ERY and PEN was examined by ermB and mefE duplex-PCR and pbp2b RFLP analysis, respectively. Clonal identity was proven by PFGE. One isolate per clone was analysed by MLST.

Results: Of the 595 SP included, 14.1%, 27.6% and 8,1% were resistant to ERY, TET and SXT, respectively. 18% were PEN intermediate. With 16 and 17 isolates two multiresistant clones (I and II) dominated in ERY resistance. Clone I resistant to ERY, TET and SXT and intermediate to PEN harboured ermB and had unique pbp2b RFLP and PFGE patterns. At three centers in Eastern Germany clone I was found. Clone II was resistant to ERY, TET and SXT, susceptible to PEN, harboured ermB and had a pbp2b RFLP pattern that was typical for PEN susceptible isolates. Its PFGE pattern was unique and was found in two centers in Eastern Germany and one center in Southern Germany. The MLST database proved that clone I and II were of international importance. Clone I had sequence type (ST) 135 and was found three times in Spain. Clone II whose ST was 273 was detected once in Greece, Iceland and Israel, three times in Portugal and twice in Italy. Moreover, clone II, also designated Mediterranean Clone, was related to the Spain-6B-2 clone.

Conclusion: Our results underline the strong impact of a few widely spread multiresistant genetic complexes on resistance in SP at local as well as at global level. In consideration of this phenomenon one should carefully choose options for the therapy of pneumococcal infections to not select multiresistant SP clones. In Germany relatively low resistance may be due to the eradication of - also internationally spread - multiresistant clones by high dosed penicillins, however PEN susceptible but ERY resistant strains may be selected by the increasing use of macrolides.

INTRODUCTION and PURPOSE

The emergence of multiresistant *S. pneumoniae* strains has become a global concern. Molecular typing studies have shown that a number of multiresistant clones have achieved significant spread within individual countries and across international boundaries. By international comparison resistance in *S. pneumoniae* is low in Germany. In a surveillance study conducted by Sahm et. al. resistance rates to penicillin were 24.7% and 33.9% in Spain and France, respectively [1]. In Germany surveillance studies have revealed penicillin-resistance rates to be less than 1%, whereas 6.3% of isolates with intermediate resistance level have been observed [2]. Macrolide resistance seems to be an increasing problem in Germany. Resistance rates of invasive pneumococci have risen from 3% to 15% in the period 1992-2000 [3]. In Spain and France the resistance rates to macrolides are with 36.9% and 57% much higher than in Germany [1].

The aim of our study was to investigate whether in Germany, despite low resistance rates, internationally spread multiresistant *S. pneumoniae* clones occur in isolates recovered from outpatients with RTIs.

MATERIALS and METHODS

A surveillance project was performed in Germany during the winter season 2000-2001. The sampling period was from November 2000 to May 2001. Seventeen centers were requested to collect clinical isolates from outpatients with RTIs (figure 2). Duplicate strains were not accepted.
MICs were determined in the central reference laboratory with a broth microdilution method following NCCLS. Antimicrobial agents tested were erythromycin A (ERY), clindamycin (CLI), penicillin G (PEN), tetracycline (TET), and co-trimoxazole (SXT). Resistance to ERY was examined by ermB and mefE duplex-PCR [4]. Clonal identity was investigated using pbp2b restriction fragment lengh polymorphism (RFLP) and pulsed-field gel electrophoresis (PFGE) [5, 6].
Multilocus sequence typing (MLST) was performed with one isolate per clone as discribed by Enright and Spratt [7]. Briefly, internal fragments from the following seven housekeeping genes were amplified: aroE (shikimate dehydrogenase) gdh (glucose-6-phosphate dehydrogenase) gki (glucose kinase) recP (transketolase) spi (signal peptidase I) xpt (xanthine phosphoribosyltransferase) and ddl (D-alanine-D-alanine ligase). After sequencing of these fragments on both strands the sequences were compared with those included in the MLST database (www.mlst.net). Each isolate could be ascribed to a known sequence type (ST). These STs were compared with all STs in the pneumococcal MLST database.

RESULTS

Table 1. Rates of susceptible, intermediate and resistant *S. pneumoniae* (n=595)

antimicrobial agent	%		
	S	I	R
ERY	85,9	0	14,1
CLI	87,2	0	12,8
PEN	82,0	18,0	0
TET	72,1	0,3	27,6
SXT	78,6	13,3	8,1

Figure 1. Distribution of ERY-susceptible and ERY-resistant isolates of *S. pneumoniae* by center

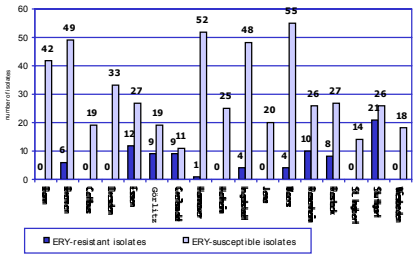

Figure 2. Spread of two multiresistant *S. pneumoniae* clones[a] dominating in ERY resistance in Germany

[a] Numbers in circles represent isolates of clone I, numbers in boxes represent isolates of clone II.

Table 2. Characteristics of two multiresistant *S. pneumoniae* clones dominating in ERY resistance in Germany

		Clone I	Clone II
number of isolates		16	17
Resistance pattern	ERY	R	R
	CLI	R	R
	PEN	I	S
	TET	R	R
	SXT	R	R
Macrolide resistance genes	ermB	+	+
	mefA	-	-
Clonal identity	pbp2b RFLP	a	b
	PFGE	1	2
	MLST ST	135	273

Among the 595 *S. pneumoniae*, the resistance rates were 14.1%, 27.6% and 8,1% to ERY, TET and SXT, respectively (Table 1). 18% were PEN intermediate. Using ERY as a reference drug, macrolide resistance varied markedly between centers. The local resistance-rates ranged from 0% to 45% (Figure 1). Two multiresistant clones (I and II) dominated in ERY resistance with significant influence on local resistance rates (Figure 2). As shown in Table 2 both clones were resistant to ERY, CLI, TET and SXT. Additionally, clone I was intermediate to PEN . Clone I and II harboured the macrolide resistance gene ermB. Each clone had a unique pbp2b-RFLP and PFGE pattern, whereby the pbp2b-RFLP pattern of clone II was typical for PEN-susceptible isolates. The spread of both clones in Germany is depicted in Figure 2. As summarized in Table 3 a MLST-database query proved that clone I and II were of international importance. Clone I had sequence type (ST) 135 and was found three times in Spain. Clone II also designated as Mediterranean Clone (ST 273) has been detected once in Greece, Iceland and Israel, three times in Portugal and twice in Italy. In the MLST-database several clones from all over the world are registered that are related to clone II. The most prominent of those is the widely spread Spain-6B-2 clone.

CONCLUSION

Also in countries like Germany with relatively low overall resistance rates internationally spread clones can be detected and show significant influence on local resistance rates. Our results underline the strong impact of a few widely spread, related clones on resistance in *S. pneumoniae* at local as well as at global level. In consideration of this phenomenon one should carefully choose options for the therapy of pneumococcal infections to not select multiresistant *S. pneumoniae* clones. A reason for the relatively low resistance in Germany may be the eradication of - also internationally spread - multiresistant clones by high dosed penicillins. However, PEN-susceptible but ERY-resistant clones may be selected by the increasing use of macrolides.

Table 3. MLST database query (www.mlst.net) for two multiresistant *S. pneumoniae* clones dominating in ERY resistance in Germany

clone		Allelic profile[b]							ST	country	designation	Number of isolates
		aroE	gdh	gki	recP	spi	xpt	ddl				
I	7 matches	7	5	4	12	6	20	46	135	Spain		3
	6 matches	7	5	4	**1**	6	20	46	136	Spain		1
		7	5	4	12	**19**	20	46	937	Spain		1
	5 matches	-	-	-	-	-	-	-	-	-	-	-
II	7 matches	5	6	1	2	6	1	14	273	Greece	Mediterranean clone	1
										Iceland		1
										Israel		1
										Portugal		3
										Italy		2
	6 matches	5	6	1	2	6	1	**28**	238	Finland		1
		7	6	1	2	6	1	14	385	Alaska		3
		5	6	1	2	**5**	1	14	895	Portugal		1
		5	6	1	2	6	1	**27**	963	Finland		1
	5 matches	5	6	1	2	6	**3**	**4**	90	Spain	Spain6B 2	13
										Australia		10
										Netherlands		3
										USA		1
										Iceland		4
										Taiwan		1
		5	6	1	2	6	**3**	**48**	91	Spain		1
		5	6	1	2	6	**3**	**54**	94	Spain		1
		7	6	1	2	6	**15**	14	146	UK		1
		7	6	1	2	6	1	**23**	147	UK		1
		5	6	**43**	2	6	1	**28**	270	Finland	Finland6B 12	2
		7	6	1	2	6	**71**	14	401	UK		1
		7	6	1	2	6	**59**	14	464	UK		1
		15	**17**	1	2	6	1	14	506	Finland		1
		1	6	1	2	**75**	1	14	885	Portugal		1
		5	**75**	1	2	6	1	**28**	1072	Finland		1
		7	6	1	2	6	1	**135**	1127	Argentina		1
		7	6	1	2	6	**133**	14	1128	Colombia		1
		5	6	1	2	6	**3**	**142**	1185	Mexico		1
		5	6	1	**1**	6	**6**	14	1224	Portugal		1

[b] Allels that differ from those found in the original profile are in boldface type.

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