

## Emergence of a Unique Multiply-Antibiotic-Resistant *Streptococcus pneumoniae* Serotype 7B Clone in Dhaka, Bangladesh<sup>†</sup>

*Streptococcus pneumoniae* is a frequent cause of potentially life-threatening infections, such as pneumonia, meningitis, and bacteremia (14). However, the global emergence of antimicrobial resistance in *S. pneumoniae* is a serious concern (4). Recent data from 12 Asian countries showed high resistance rates (17, 18). We studied prospective resistance to a large number of antimicrobial agents in pneumococcal isolates. We also analyzed macrolide resistance, with an emphasis on resistance genes, molecular epidemiology, and serotype patterns.

From 1999 to 2002, *S. pneumoniae* isolates ( $n = 136$ ) from blood and cerebrospinal fluid (CSF) ( $n = 60$ ) and nasopharynx ( $n = 76$ ) of children (<5 years) with pneumonia and meningitis from three hospitals in Dhaka, Bangladesh, were studied. MICs were determined by CLSI broth microdilution method (1) and by Etest (AB Biodisk, Solna, Sweden). Macrolide resistance phenotypes were determined by triple-disk test (11, 12) and macrolide resistance genotypes by a light cycler protocol (15). Isolates were serotyped by antisera (Statens Serum Institut, Copenhagen, Denmark). Multilocus sequence typing (MLST) was carried out (2), and two predominant sequence types (ST) were analyzed by use of the eBURST2 program (3).

MIC results for *S. pneumoniae* (Table 1) showed high rates of resistance to sulfamethoxazole-trimethoprim (77.9%) and tetracycline (46.3%). The resistance rates of other drugs were low. The rates of multiply-resistant isolates were 27.9% and 11.7% against 2 and  $\geq 3$  classes of antibiotics, respectively. Six (4.4%) isolates (Table 2) were resistant to erythromycin A; five of them exhibited the partially inducible iMcLS<sub>B</sub> phenotype (susceptible or intermediate to rokitamycin but developing no induction resistance to rokitamycin in the presence of erythromycin) and one strain the M phenotype (resistant to erythromycin, azithromycin, and clarithromycin but susceptible to clindamycin and streptogramin B). Isolates with the iMcLS<sub>B</sub> phenotype were positive for the *erm*(B) gene, and the isolate displaying the M phenotype was positive for *mef*(A). Macrolide-resistant isolates were serotypes 7B ( $n = 4$ ), 9V ( $n = 1$ ), and 18C ( $n = 1$ ). MLST results of serotype 7B macrolide-resistant strains established two sequence types: ST 1553 (strains 14, 39, and 94) and ST 1586 (strain 61). ST 1586 is a single-locus variant (SLV) of ST 1553, and both appear to belong to a single clonal complex. In addition, one isolate was a serotype 9V variant of ST 1553 (strain 68), indicating serotype switching. The *mef*(A)-positive strain was not closely ge-

TABLE 1. MIC ranges, MIC<sub>50</sub>s, and MIC<sub>90</sub>s of 136 *S. pneumoniae* strains isolated in Bangladesh

Antibiotic <sup>a</sup>	MIC (μg/ml)			% Resistant
	Range	MIC <sub>50</sub>	MIC <sub>90</sub>	
Penicillin G	0.016–0.5	0.016	0.03	8.8
Amoxicillin	0.016–0.5	0.016	0.06	0
Cefotaxime	0.016–0.25	0.016	0.06	0
Gatifloxacin	0.016–1	0.25	0.25	0
Ciprofloxacin	0.06–>32	1	1	2.9
Teicoplanin	0.016–0.125	0.06	0.06	0
Vancomycin	0.03–0.25	0.25	0.25	0
Telithromycin	0.016–0.06	0.016	0.016	0
Tetracycline	0.125–>32	0.5	>32	46.3
Clindamycin	0.06–>32	0.06	0.06	3.7
Erythromycin A	0.06–>32	0.06	0.06	4.4
SXT*	0.03–>32	4	6	77.9
Chloramphenicol*	1–6	2	2	8.1

<sup>a</sup> SXT, sulfamethoxazole-trimethoprim. \*, MICs were determined by Etest (see text). For ciprofloxacin, a breakpoint of >4 μg/ml was used.

netically related to this clonal complex (strain 28, ST 113). eBURST analysis (Fig. 1) showed ST 1553 and ST 1586 to form a pair of SLVs. No other SLVs were found in the MLST database (www.mlst.net). An analysis for double-locus variants determined these two ST to be members of a group of 26 ST represented by 37 isolates with ST 230 as the predicted group founder. ST 230 is represented in the MLST database by two erythromycin-sensitive serotype 14 isolates from Denmark and Italy and an erythromycin-resistant serotype 24F isolate, also from Italy. Six ST in the group are double-locus variants of either ST 1586 or ST 1553 or both.

Our study highlights a high level of tetracycline and sulfamethoxazole-trimethoprim resistance in Bangladesh. There is increasing concern over resistance in pneumococci to sulfamethoxazole-trimethoprim, which is recommended by the WHO as a first-line drug for treating nonsevere pneumonia. Our study supports the view that this recommendation may not be optimal for Bangladesh; however, changing to alternative agents, such as amoxicillin, is costly ([http://www.who.int/child-adolescent-health/publications/referral\\_care/chap3/chap31.htm](http://www.who.int/child-adolescent-health/publications/referral_care/chap3/chap31.htm)). Moreover, the widespread use of sulfamethoxazole-trimethoprim may further drive the spread of multiply-resistant pneumococcal clones and

TABLE 2. Characteristics of macrolide-resistant *S. pneumoniae* strains isolated from children in Bangladesh<sup>a</sup>

Strain	Source	MIC (μg/ml)						Macrolide resistance			
		ERY	CLI	PEN	SXT	TET	CHL	Genotype	Phenotype	Serotype	ST <sup>b</sup>
14	CSF	≥256	>32	0.25	16	16	2	<i>erm</i> (B)	iMcLS <sub>B</sub>	7B	1553
39	Blood	≥256	>32	0.5	16	>32	2	<i>erm</i> (B)	iMcLS <sub>B</sub>	7B	1553
61	NP	≥256	>32	0.5	16	>32	2	<i>erm</i> (B)	iMcLS <sub>B</sub>	7B	1586
68	NP	≥256	>32	0.25	>32	>32	2	<i>erm</i> (B)	iMcLS <sub>B</sub>	9V	1553
94	NP	≥256	16	0.5	8	1	2	<i>erm</i> (B)	iMcLS <sub>B</sub>	7B	1553
28	CSF	1	0.06	0.03	0.5	8	2	<i>mef</i> (A)	M	18C	113

<sup>a</sup> Abbreviations: NP, nasopharynx; ERY, erythromycin A; CLI, clindamycin; PEN, penicillin G; SXT, sulfamethoxazole-trimethoprim; TET, tetracycline; CHL, chloramphenicol.

<sup>b</sup> ST 1553 showed alleles 6, 5, 2, 17, 6, 22, and 14; ST 1586 showed alleles 43, 5, 2, 17, 6, 22, and 14; and ST 133 showed alleles 7, 2, 1, 1, 10, 1, and 21.

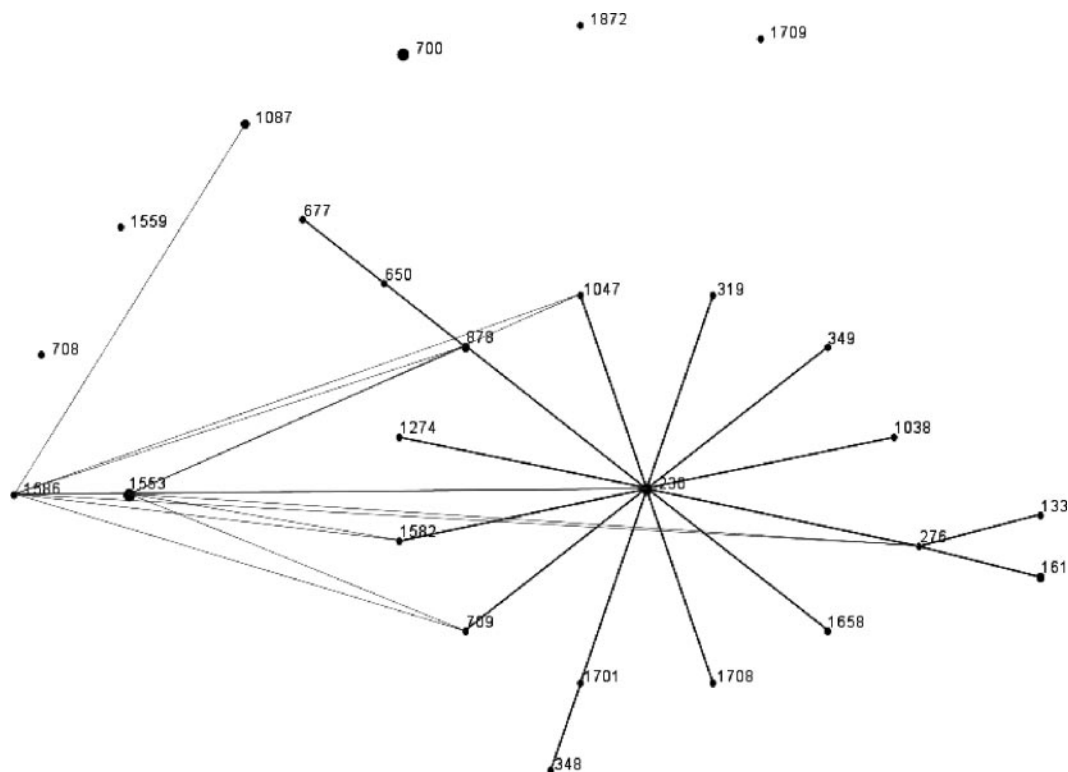


FIG. 1. eBURST analysis of ST of two multiply-resistant serotype 7B strains in this study (ST 1586 and ST 1553) and 2,681 different ST of *S. pneumoniae* available in the MLST database ([www.mlst.net](http://www.mlst.net)). Single-locus variants are connected by black lines. Double-locus variants are not connected by lines, with the exception of the double-locus variants of ST 1553 and ST 1586, which are connected by gray lines.

may also select resistance to penicillin G, chloramphenicol, and macrolides. Our observation of a high level of resistance to sulfamethoxazole-trimethoprim in Bangladesh is consistent with findings from the mid-1990s (16). In contrast, the rates of resistance to penicillin G, macrolides, and other drugs are relatively low compared to those described in recent reports from other Asian countries, except India (8, 17, 18). Multiply-resistant *S. pneumoniae* is a problem in Bangladesh. Although an increasing trend of fluoroquinolone resistance has been reported in Hong Kong, Spain, and Canada (7, 9), a low rate (2.9%) of ciprofloxacin resistance was observed in our study. However, gatifloxacin and moxifloxacin remained active in vitro, indicating their potential utility in Bangladesh.

The predominance of the MLS<sub>B</sub> phenotype (resistance to all macrolides, lincosamides, and streptogramin B)/*erm*(B) genotype in our study is consistent with recent findings from Sri Lanka, Korea, China, Taiwan, Japan, Spain, Italy, France, and South Africa (6, 9, 15, 17). All strains with the MLS<sub>B</sub> phenotype exhibited a partially macrolide-inducible iMLS<sub>B</sub> phenotype, i.e., they were susceptible or had intermediate resistance to rokitamycin and had no resistance induction to rokitamycin in the presence of erythromycin (13). This phenotype is the most common mechanism of macrolide resistance in Bangladesh, although the sample size is small. Of note, the majority of macrolide-resistant strains belong to a single serotype, 7B. Serotype 7B infections were recorded only in India in the late 1990s (5). Three 7B isolates and the 9V variant have the same sequence type, ST 1553, and form a clonal complex. In addition, one 7B isolate is a single-locus (*aroE*) variant (ST 1586) of ST 1553. Of note, ST 1553 and ST 1586 are not closely related to any other clone in the MLST database, as shown by

eBURST analysis. The strain exhibiting a *mef* genotype belongs to ST 113. Strains of this clone (global clone 36 of the Pneumococcal Molecular Epidemiology Network [<http://www.mlst.net>; <http://www.sph.emory.edu/PMEN>]) (10) are serotype 18C and were isolated from meningitis in The Netherlands, the United Kingdom, and Spain in the 1980s and 1990s. Interestingly, the present report is the first to document macrolide resistance in an isolate of this particular clone.

In summary, our report shows that resistance to beta-lactams, macrolides, and fluoroquinolones in pneumococci is not as yet a serious problem in Bangladesh, unlike in many other Asian countries. However, the emergence of a unique multiply-resistant serotype 7B clone reiterates the need for continual surveillance of antimicrobial resistance in pneumococci.

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