original article

Impact of the revised penicillin susceptibility breakpoints for *Streptococcus pneumoniae* on antimicrobial resistance rates of meningeal and non-meningeal pneumococcal strains

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BACKGROUND AND OBJECTIVES: In January 2008, the Clinical Laboratory Standard Institute (CLSI) revised the *Streptococcus pneumoniae* breakpoints for penicillin to define the susceptibility of meningeal and non-meningeal isolates. We studied the impact of these changes. In addition, the pneumococcal resistance rate to other antimicrobial agents was reviewed.

DESIGN AND SETTING: Laboratory data on peumococcal isolates collected retrospectively from hospitalized children in tertiary care hospital in Riyadh, Saudi Arabia from January 2006 to March 2012.

PATIENTS AND METHODS: Only sterile samples were included from cerebrospinal fluids, blood, sterile body fluids and surgical tissue. Other samples such as sputum and non sterile samples were excluded. We included samples from children 14 years old or younger. The minimum inhibitory concentration (MIC) for penicillin, cefuroxime, ceftriaxone and meropenem were determined by using the E-test, while susceptibility to erythromycin, cotrimoxazole and vancomycin were measured using the disc diffusion methods following the guideline of CLSI.

RESULTS: Specimens were analyzed in two different periods: from January 2006 to December 2007 and from January 2008 to March 2012. During the two periods there were 208 samples of which 203 were blood samples. Full penicillin resistance was detected in 6.6% in the first period. There was decrease in penicillin nonmeningeal resistance to 1.5% and an increase in resistance in penicillin meningeal 68.2% in the second period (P=.0001). There was an increase in rate of resistance among *S pneumoniae* isolates over the two periods to parenteral cefuroxime, erythromycin and cotrimoxazole by 34.6%, 35.5% and 51.9%, respectively. Total meropenem resistance found 4.3% and no vancomycin resistance was detected.

CONCLUSIONS: The current study supports the use of the revised CLSI susceptibility breakpoints that promote using penicillin to treat nonmeningeal pneumococcal disease, and might slow the development of resistance to broader-spectrum antibiotics.

Streptococcus pneumoniae remains a major cause of morbidity and mortality worldwide in spite of recent advances in antimicrobial therapy and vaccine development. It is a leading cause of pneumonia, pyogenic meningitis, bacteremia and other infections.[1] The emergence of multiple drug resistance has complicated the empirical treatment of pneumococcal infection.[2] Incidence of invasive pneumococcal diseases was more prevalent in children younger than 5 years of age and elderly of more than 65 years of age.[3] Despite the importance given to penicillin non-susceptibility,[1,4-6] it was found that there is no increase in mortality in patients with pneumococcal pneumonia.[7,8]

In January 2008, the Clinical and Laboratory Standards Institute (CLSI) published revised breakpoints for susceptibility when testing penicillin against *Streptococcus pneumoniae*. In March 2008, the United States (US) Food and Drug Administration susceptibility breakpoints for penicillin versus *S pneumoniae* were similarly revised via changes in the penicillin

package insert for the primary generic manufacturer. The comparison between pre-2008 and the new 2008 revised breakpoints for meningitis, nonmeningitis intravenous and oral administration can be seen in **Table 1 and 2.** Because capillaries and pulmonary alveoli are separated by no more than the thickness of two cells and a shared basement membrane, penicillin concentrations in the alveoli tend to approach those in the blood. A redefinition of pneumococcal susceptibility in otitis media and sinusitis was necessary since these tissues are highly vascular and lack a tight endothelial junction, unlike the blood-brain barrier.[8] The impact of the new definition on the epidemiology of penicillin resistance has not been thoroughly evaluated;[8] the objective of our study was to compare the difference over time in *S pneumoniae* penicillin susceptibility rates when applying the revised CLSI breakpoints.

PATIENTS AND METHODS

From January 2006 to March 2012, antimicrobial sensitivity testing data on different isolates of *S pneumoniae* were retrospectively collected through the Microbiology Laboratory at King Faisal Specialist Hospital and Research Centre in Riyadh, Saudi Arabia. Two categories of clinical specimen source were analyzed and included: cerebrospinal fluid (for meningitis syndrome), blood and sterile isolates of different body fluids and surgical tissues (for nonmeningitis syndrome). Nonsterile isolates were excluded. Samples were from children less than 14 years old that were categorized into 3 groups: younger than 2 years old, 2-5 years old and 5-14 years old. Two time periods were considered in the analysis: (1) 2006 to 2008; (2) 2008-2012.

S pneumoniae was identified by use of standard methods. Antibiotic susceptibility testing was determined by means of the E-test (AB Biodisk, Solna, Sweden) according to the manufacturer's instructions.

Table 1. Former and current Clinical and Laboratory Standard Institute susceptibility breakpoints for penicillin for treatment of *Streptococcus pneumoniae* infection.

Period, syndrome and route of administration	MIC µg/mL, by susceptibility category		
	Susceptible	Intermediate	Resistant
Before January 2008	< 0.06	0.12- 1	>2
After January 2008 to present			
For meningitis via intravenous route	< 0.06	None	> 0.12
For nonmeningitis syndrome			
Via intravenous administration	< 2	4	>8
Via oral administration	< 0.06	0.12- 1	>2

MIC: Minimum Inhibitory Concentration

Table 2. CLSI interpretive breakpoints (MIC) for Streptococcus pneumonia for selected antibiotics Data from: Reference CLSI. M100-S18.200

Cefuroxime MIC µg/ml			
Parenteral	<0.5	1	>2
Oral	<1	2	>4
Cefotaxime, Ceftriaxone MIC µg/ml			
Meningeal	<0.5	1	>2
Nonmeningeal	<1	2	>4
Meropenem			
<0.25	0.5	>1	

The minimal inhibitory concentration (MIC) was determined for penicillin, cefuroxime and ceftriaxone using the E-test (AB Biodisk) strip placed onto blood agar (Oxoid Mueller-Hinton base with 5% sheep blood). The Kirby-Bauer disc diffusion susceptibility test was done according to CLSI established methods for erythromycin, trimethoprim-suphamethazole and vancomycin antibiotics.

Pre-2008 and 2008 CLSI breakpoints were used for the categorization of penicillin resistance as shown in **Table 1.** Vancomycin susceptible with disc diffusion ≥17 mm, whereas erythromycin and trimethoprim-suphamethazole were considered susceptible, intermediate and resistant with the following readings: ≥21 mm, 16-20 mm and ≤15 mm, respectively, for erythromycin and ≥19mm, 16-18 mm and ≤15 mm respectively for trimethoprim-suphamethazole.

The levels and trends in antibiotics susceptibility, intermediate, and resistant are presented as annual prevalence plus minus a 95% CI and were compared between the 2 time periods (margin of error 3%). Statistical analysis was done using SPSS version 19. Continuous variables were repeated as mean and standard. Categorical variables were reported as percentages. The chi-square test was used to assess the associations.

RESULTS

During the two studied periods there were 208 samples, 76 samples in period 1 (January 2006-December 2007) and 132 samples in period 2 (January 2008-March 2012). The source of the isolates was blood in 203 samples and five other samples included: 2 cerebrospinal fluid, 1 pleural fluid and 2 surgical tissues. There were 111 males (53.4%) and 97 females (46.6%). Patient ages ranged from 3 months and 14 years (mean [SD] age 7.3 [3.38] years). The number of isolates according to the age group was 20 in children less than 2 years

Table 3. Penicillin resistance over the 2 periods according to age, gender and sample source.

	Period 1			Period 2								
	Penicillin n=72			Oral penicillin n=115			Penicillin nonmening n=125			Penicillin mening. n=120		
	FR (%)	IR (%)	*P*	FR (%)	IR (%)	*P*	FR (%)	IR (%)	*P*	FR (%)	IR (%)	*P*
Age (years)												
<2	1 (5)	1 (5)		2 (10)	9 (45)		0 (.0)	0 (.0)		11 (55)	0	
2-5	1 (2.2)	4(8.7)	.004	2 (4.3)	25 (54.3)	.052	1 (2.2)	0 (.0)	.015	30 (65.2)	0	.003
5.-14	3 (2.1)	42 (29.6)		6 (4.2)	42 (29.6)		1 (.7)	1 (.7)		49 (34.5)	0	
Gender												
Male	2 (1.8)	25 (22.5)	.782	7 (6.3)	38 (34.2)	.616	0 (.0)	1 (.9)	.339	49 (44.1)	0	.840
Female	3 (3.1)	22 (22.7)		3 (3.1)	38 (39.2)		2 (2.1)	0 (.0)		41 (42.3)	0	
Source												
Blood	5 (7.35)	45 (66.18)	0.549	10 (8.7)	76 (66.09)		2 (1.6)	1 (.8)	.987	90 (75.63)	0	0.82
Others, CSF Pleural Tissue	0 (0.0)	2 (50)		0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)		0 (0.0)	0	0

FR full Resistance, IR Intermediate Resistance; N=number of isolates

old, 46 from 2 to 5 years old and 142 between 5 and 14 years old. According to the age group, there was increasing in prevalence of *S pneumoniae* isolates in older than 5 years of age with 68.3% and the least were among less than 2 years old (9.6%). Penicillin resistance was more among children older than 5 years old (31.7%) in period 1, whereas in period 2, resistance was observed more frequent in age group 2 to 5 years old with statistical significance in parenteral penicillin. There was no difference between the two sexes in penicillin resistance pattern (**Table 3**). There was no statistical difference in penicillin resistance according to specimen type. Other than blood samples, there were 2 samples (50%) (CSF and pleural fluids) that developed penicillin resistance in period 1 and none in period 2. The full penicillin resistance was 6.6% before applying the revised CLSI criteria showing increased resistance in penicillin meningeal (68.2%) and decreased resistance in nonmeningeal (1.5%) (*P*=.0001) (**Table 4**). In oral penicillin, there was increase in susceptibility (22.0%) (*P*=.0001). There was increase in the rate of resistance among pneumococcal isolates between the two periods to parenteral cefuroxime, erythromycin and trimethoprim-suphamethazole (*P*=.0001) (**Table 5**). There was no difference in the rate of resistance to cefotaxime/ceftriaxone (meningeal and nonmeningeal) and meropenem in the two periods. No resistant isolates among CSF, pleural fluids and surgical tissues were detected to cefotaxime/ceftriaxone, whereas there was one resistance sample among pleural fluids to meropenem. The total number of resistant isolates to meropenem in the two periods were 9 (4.3%)(three samples full resistance and six were intermediate resistance); all the resistance isolates were either resistant or intermediate to penicillin in period 1 whereas in period 2 there were 3 samples intermediate to meropenem but susceptible to penicillin nonmeningeal. All isolates which were resistant to meropenem were resistant to cefotaxime/ceftriaxone meningeal except one isolate was resistant to meropenem but susceptible to cefotaxime/ceftriaxone and 6 samples were susceptible to cefotaxime/ceftriaxone non-meningeal.

Table 4 The susceptibility pattern of penicillin over the 2 periods.

	Period 1	Period 2		
	Penicillin %	Oral penicillin %	Penicillin nonmeningeal %	Penicillin meningeal %
Susceptible	26.3	22	94.2	22.7
Intermediate	61.8	57.6	0.8	0
Resistant	6.6	7.6	1.5	68.2
P value	.0001	.0001	.0001	.0001

There was no detected resistance to vancomycin in our study (**Table 5**).

DISCUSSION

Based on the old CLSI penicillin resistance breakpoint (≥2 μg/mL) resistance was low (6.6%), when comparing this with the international data, penicillin resistance

Table 5. Resistant isolates of different antimicrobial agents over the 2 periods.

	Resistant isolates			
	Period 1 n (%)	Period 2 n (%)	Total n (%)	*P*
Oral cefuroxime n=126	-	48 (36.4)	48 (23.0)	.0001
Parental cefuroxime n=137	8 (10.5)	64 (48.5)	72 (34.5)	.0001
Cefotaxime/ceftriaxone Nonmeningeal n=204	0 (.0)	4 (3.1)	4 (1.9)	.455
Cefotaxime/ceftriaxone Meningeal n=188	16 (21.1)	23 (17.4)	39 (18.8)	.543
Meropenem n=195	4 (5.2)	5 (3.8)	9 (4.3)	.771
Vancomycin n=197	0 (.0)	0 (.0)	0 (.0)	
Erythromycin n=144	12 (15.8)	62 (47)	74 (35.5)	.0001
SXT n=143	15 (19.7)	93 (70.5)	108 (51.9)	.0001

which had an initial rise that started 1996, peaked in 2000, declined until 2003, and rebound through 2008 (15.6%, 23.2%, 15.4% and 16.9%,respectively).[8] Our national studies in Saudi Arabia showed a variance in rate of penicillin resistance with no penicillin resistance, but 14.7% intermediate resistance in 1995,[9] 14.9% resistance in 2000,[10] and in 2001 among 34% of resistant isolates there were 97% with intermediate resistance.[5] Penicillin resistance is more in children more than 2 years old which is not compatible with other studies done between 1999 to 2009 with more resistance in in children younger than 2 years old.[11] When applying the revised CLSI criteria of in vitro susceptibility to penicillin, the rate of resistance to parenteral penicillin for nonmeningeal infections decrease into 1.5%. This was expected as the revised susceptibility breakpoints increased to 2 μg/mL for nonmeningitis infections. These changes were based on retrospective[12] and prospective[13] studies involving adults, as well as studies involving children,[14] which demonstrated that the outcomes of pneumococcal pneumonia caused by penicillin nonsusceptible strains were no different in patients treated with parenteral penicillin than in patients treated with other agents, suggesting that the susceptibility breakpoints established for meningitis (≤0.06 μg/mL) did not apply to pneumonia.[15] Our results were compatible with the finding by the Active Bacterial Core surveillance (ABCs), 2006-2007 with resistance rate in *S pneumoniae* isolates to penicillin nonmeningeal was 1.2%.[16] There were two other studies, one done by Mera et al, [8] where they analyzed 97 843 US isolates from the surveillance network database from period 1995-2008 comparing penicillin resistance using the old and then the revised CLSI criteria, they found increase in resistance in nonmeningeal isolates to 1.52% in 2008 compared to 0.24% in 2003. The second study that dealt with the revised CLSI criteria was a Brazilian study[17] where they isolated strains from blood and pleural fluids in pediatric patients less than 12 years old and got only one isolate with intermediate resistance to penicillin. There was increase in resistance for parenteral penicillin for meningitis infection to 68.2% after applying the resistant breakpoints ≥0.12, which is higher than found by ABCs data 27.5% and data by Mera et al.[8] 34.8%. Although there were no changes in penicillin breakpoints in oral penicillin in the revised CLSI criteria, resistance increase from 6.6% to 7.6%, but Mera et al found no change compared to old criteria with resistance 23.8%.

Among cephalosporin, there is an increase in cefotaxime/ceftriaxone nonmeningeal resistance to 1.9% of isolates, which is still low compared to published data. Previous studies showed 6% resistance to 3rd generation cephalosporins in 1995,[6] 13% in 2004 onward.[18] In Saudi Arabia ceftriaxone resistance was low, 2 isolates (1.3%) in 1995[9] and one isolate in 2001,[5] but Memish et al found ceftriaxone resistance in 14.9% of isolates in three major hospital in Saudi Arabia in 2000.[10]

There was a marked increase in the resistance rate of erythromycin and trimethoprim-suphamethazole (35.5% and 51.9%, respectively) which is higher compared to reported data.[19,20] Meropenem resistance remained low in our population (4.3% compared to 26% reported among 53 isolates from blood and CSF from July 1998 to August 1999).[21] This study support the published studies before 2008[12-14] evaluating penicillin as monotherapy for treatment during the first 48 hours of nonmeningitis pneumococcal infections and shows

the impact of the revised CLSI criteria in decreasing the reported numbers of penicillin resistant among pneumococcal isolates. The changes in penicillin breakpoints for *S pneumoniae* have the potential to allow clinicians to increase use of penicillin to treat penicillin-susceptible nonmeningeal pneumococcal infections, instead of using broader-spectrum antimicrobials.

Because most antimicrobial reports from clinical laboratories have included only one set of susceptibility breakpoints, the use of multiple sets of breakpoints has the potential to cause confusion among clinicians. Thus clinicians should review all susceptibility results, decide which set of breakpoints to use, based on the patient's clinical presentation and the planned route of of drug administration.

Although it had good numbers of samples, in our study there were only two CSF samples, which did not reflecting well the meningitis infections although the new meningitis breakpoints were applied to the blood isolates as well. In addition, this study was limited to a tertiary hospital in Riyadh.

In conclusion, the initial treatment for most non-meningeal pneumococcal infections remains being empirical in terms of etiology and susceptibility to drugs. After the detection of the etiological agent, antimicrobial susceptibility testing influences clinicians' antibiotic choices. Our current study supports the use of the revised CLSI susceptibility breakpoints, which will promote using narrower-spectrum antibiotics to treat non-meningeal pneumococcal disease, and might slow the development of resistance to broader-spectrum antibiotics.

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