# Pilot Studies to Guide CLSI Re-evaluations of *S. pneumoniae* and β-haemolytic Streptococci Disk Diffusion Criteria for Commonly Used Penicillins and Cephalosporins

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## ABSTRACT

**Background**: CLSI β-lactam (β-L) disk diffusion (DD; Kirby-Bauer) methods have no correlate zone for the old or new susceptible (S) breakpoints for SPN, and β-haemolytic streptococci (BHS)-S criteria have been guided by penicillin (PEN)-S results. With the recently modified PEN-S criteria (≤2 μg/ml) for high-dose pneumococcal (SPN) pneumonia therapy, the DD test was re-examined using alternative DD contents for 3 agents and X-S for 12 β-L drugs.

**Methods**: 153 SPN and 50 BHS were selected from year 2008 isolates to represent all relevant wildtype and S or R subsets. PEN (1-, 2-, 10-U), cefotaxime (CTX; 5-, 30- μg), ceftriaxone (CRO; 5-, 30- μg) were tested with several candidate disk contents, and amoxicillin/clavulanate (AUG) and cefepime (FEP) were also tested. BHS were processed by X-S analyses to PEN by MIC and zone criteria (12 β-lactams). All tests used CLSI M02-A10, M07-A8 (2009) methods and M100-S19 criteria.

**Results**: BHS X-S results indicate PEN (MIC, ≤0.12 μg) continues to predict high activity (MIC, ≤0.25 μg/ml) for AUG, CTX, CRO, FEP, cephalothin and cefuroxime, but not cephalexin (MIC range to 4 μg/ml), cefadroxil (2 μg/ml), cefaclor (1 μg/ml) and ceftazidime (2 μg/ml). SPN PEN X-S analyses at ≤2 μg/ml favorably correlated with CTX, CRO, FEP and AUG activity. DD testing could be applied with acceptably low error rates for 5 commonly used β-lactams (Table), and X-S was without serious false-S or false-resistance error (86.9-92.2% absolute categorical agreement).

**Table:** Examples of documented acceptable DD-S breakpoint criteria when testing SPN against five popular antimicrobials.

| Agent | Disk conc. (U or μg) | S Criteria | | Error rates (%)[a] | | | Acceptable? (%) |
|---|---|---|---|---|---|---|---|
| | | MIC (μg/ml) | DD zone (mm) | VM | Ma | Mi | |
| PEN | 1 | ≤0.06 | ≥24 | 1.3 | 0.0 | - | Yes (98.7) |
| | 2 | ≤0.06 | ≥26 | 0.6 | 2.6 | - | Yes (96.8) |
| | 10 | ≤0.06 | ≥32 | 0.6 | 2.0 | - | Yes (97.4) |
| | 10 | ≤2 | ≥19 | 0.0 | 0.0 | 7.2 | Yes (92.8) |
| CRO | 5 | ≤1 | ≥19 | 0.0 | 0.0 | 3.3 | Yes (96.7) |
| | 30 | ≤1 | ≥26 | 0.0 | 0.0 | 4.6 | Yes (95.4) |
| CTX | 5 | ≤1 | ≥19 | 0.0 | 0.0 | 4.6 | Yes (95.4) |
| | 30 | ≤1 | ≥27 | 0.0 | 0.0 | 5.2 | Yes (94.8) |
| FEP | 30 | ≤1 | ≥26 | 0.0 | 0.0 | 7.2 | Yes (92.8) |
| AUG | 30 | ≤2 | ≥22 | 0.0 | 0.0 | 5.2 | Yes (94.8) |

a. VM = very major (false-S), Ma = major (false-R) and Mi = minor.

**Conclusions:** DD testing for contemporary SPN & BHS appear practical and accurate for most widely used β-L's, demonstrating acceptable performance and potential X-S criteria use (PEN surrogate). CLSI should re-establish simple, cost effective DD criteria for these prevalent pathogens.

## INTRODUCTION

With the adoption of the Clinical and Laboratory Standards [CLSI; formerly the National Committee for Clinical Laboratory standards (NCCLS)] breakpoint tables in the 1970's, the recommendation for testing one antimicrobial agent to represent the susceptibility for other similar agents has been widely accepted (class-disk concept). One such instance is the testing of a penicillin disk (10-U) against *S. pneumoniae* and β-haemolytic streptococcal isolates to predict the susceptibility to other β-lactam agents including β-lactam/β-lactamase inhibitor combinations and carbapenems. Similarly the cephalothin (30-μg) or cefazolin (30-μg) disks have been used to predict susceptibility (not resistance) to other cephalosporin compounds, especially orally delivered agents.

With the recently adopted breakpoints for penicillin susceptibility of ≤2 μg/ml (high-dose regimes; 12 million units/day) and resistance at ≥8 μg/ml against *S. pneumoniae*, the disk diffusion test should be re-evaluated to confirm the cross-susceptibility with other β-lactam agents since no disk zone diameter breakpoints are found in the current CLSI M100-S19 tables.

## MATERIALS AND METHODS

Bacterial Isolates. A collection of 50 β-haemolytic streptococci (primarily *S. pyogenes*, 80%) and 153 *S. pneumoniae* were selected from the 2008 SENTRY Antimicrobial Resistance Surveillance study based on their susceptibility to penicillin. *S. pneumoniae* isolates were evenly distributed according to non-meningitis, oral penicillin breakpoints; susceptible at ≤0.06 μg/ml, intermediate at 0.12-1 μg/ml and resistant at ≥2 μg/ml.

Susceptibility Testing. Frozen reference broth microdilution panels with Mueller-Hinton broth supplemented with 2-5% lysed horse blood were produced by TREK Diagnostics (Cleveland, Ohio, USA) containing penicillin (PEN), ampicillin, amoxicillin/clavulanate, cefaclor, cephalexin, cefadroxil, cephalothin, cefuroxime, ceftazidime, ceftriaxone, cefotaxime, and cefepime were inoculated according to CLSI standard methods (M07-A8, 2009). Concurrently, the CLSI disk diffusion (DD) method (M02-A10, 2009) with 150mm diameter Mueller-Hinton agar plates supplemented with 5% sheep blood were used for testing a total of nine disks including penicillin (1-, 2-, 10-U), amoxicillin/clavulanate (30-μg), ceftriaxone (5-, 30-μg), cefotaxime (5-, 30-μg) and cefepime (30-μg).

## RESULTS

- Against BHS, the PEN MIC test (susceptible breakpoint at ≤0.12 μg/ml) accurately predicted high activity (MIC, ≤0.25 μg/ml) for six of the β-lactam agents tested, but not for cephalexin (MIC values ranging to 4 μg/ml), cefadroxil (range to 2 μg/ml), ceftazidime (range to 2 μg/ml) and cefaclor (range to 1 μg/ml; Table 1).
- The MIC/DD zone diameter correlations for the seven β-lactam agents tested against BHS all showed MIC values ≤0.25 μg/ml and zone diameter results ≥21 mm (Data not shown).
- The new PEN-susceptible MIC breakpoint of ≤2 μg/ml for high-dose *S. pneumoniae* therapy showed excellent predictive cross-susceptibility with cefotaxime (minor error rate only, 7.8%), cefepime (8.5%) and ceftriaxone (9.2%). Elevated unacceptable error rates were observed for ceftazidime (12.4%), amoxicillin/clavulanate (13.1%), ampicillin (13.8%), cefuroxime axetil (40.0%) and cefaclor (50.9%; Table 2).
- For the *S. pneumoniae* isolates, the MIC/DD correlation for the five β-lactam agents tested showed an acceptable susceptible predictive ability with the tested lower disk concentrations for PEN, ceftriaxone and cefotaxime, each generally performing better than current CLSI-recommended disk drug contents. Accuracy rates ranged from 92.8 to 98.7% (Table 3).
- Excellent intermethod correlations of MIC/DD zone diameter results using regression analysis for the *S. pneumoniae* tests were observed. Solid lines at proposed susceptible and resistant MIC and DD breakpoints show the limited number of strains producing error rates, only 1.3-7.2% (Table 3; Figures 1 and 2).

**Table 1**. Penicillin cross-resistance data for 50 β-haemolytic streptococci tested using CLSI broth microdilution methods against 11 β-lactam agents.

| Compound Agent | MIC (μg/ml) | Penicillin MIC (μg/ml): | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | ≤0.008 | 0.015 | 0.03 | 0.06 | 0.12 | 0.25 | % susceptible[a] |
| Ampicillin | ≤0.5 | 41 | 3 | 5 | 1 | - | - | 100.0[b] |
| | 1 | - | - | - | - | - | - | |
| Amoxicillin/clavulanate | ≤0.12 | 41 | 3 | 5 | 1 | - | - | 100.0[b] |
| | 0.25 | - | - | - | - | - | - | |
| Cephalothin | ≤0.12 | 41 | 2 | 5 | - | - | - | 100.0[b] |
| | 0.25 | 1 | 1 | - | 1 | - | - | |
| | 0.5 | - | - | - | - | - | - | |
| Cephalexin | ≤0.25 | 33 | - | - | - | - | - | -[c] |
| | 0.5 | 8 | 3 | - | - | - | - | |
| | 1 | - | - | - | - | - | - | |
| | 2 | - | - | 5[d] | - | - | - | |
| | 4 | - | - | - | 1 | - | - | |
| | 8 | - | - | - | - | - | - | |
| Cefadroxil | ≤0.25 | 41 | 3 | - | - | - | - | -[c] |
| | 0.5 | - | - | 1 | - | - | - | |
| | 1 | - | - | 3 | 1 | - | - | |
| | 2 | - | - | 1 | - | - | - | |
| | 4 | - | - | - | - | - | - | |
| Cefaclor | ≤0.12 | 41 | 2 | - | - | - | - | 100.0[b] |
| | 0.25 | - | 1 | - | - | - | - | |
| | 0.5 | - | - | 3 | - | - | - | |
| | 1 | - | - | 2 | 1 | - | - | |
| | 2 | - | - | - | - | - | - | |
| Cefuroxime | ≤0.12 | 41 | 3 | 5 | 1 | - | - | 100.0[b] |
| | 0.25 | - | - | - | - | - | - | |
| Cefotaxime | ≤0.06 | 41 | 3 | 4 | 1 | - | - | 100.0 |
| | 0.12 | - | - | 1 | - | - | - | |
| | 0.25 | - | - | - | - | - | - | |
| Ceftriaxone | ≤0.06 | 41 | 3 | 4 | 1 | - | - | 100.0 |
| | 0.12 | - | - | - | - | - | - | |
| | 0.25 | - | - | 1 | - | - | - | |
| | 0.5 | - | - | - | - | - | - | |
| Ceftazidime | ≤0.12 | 41 | - | - | - | - | - | -[c] |
| | 0.25 | - | 3 | 3 | 1 | - | - | |
| | 0.5 | - | - | 1 | - | - | - | |
| | 1 | - | - | - | - | - | - | |
| | 2 | - | - | 1 | - | - | - | |
| | 4 | - | - | - | - | - | - | |
| Cefepime | ≤0.06 | 41 | 3 | 4 | 1 | - | - | 100.0 |
| | 0.12 | - | - | - | - | - | - | |
| | 0.25 | - | - | 1 | - | - | - | |
| | 0.5 | - | - | - | - | - | - | |

a. CLSI M100-S19 breakpoint criteria were used.
b. Susceptibility rate based on penicillin MIC per CLSI recommendations.
c. No interpretative criteria provided by CLSI.
d. Boxed values may indicate non-susceptible MIC values.

**Table 2**. Penicillin cross-susceptibility data for 153 *S. pneumoniae* tested using CLSI methods against 11 β-lactam agents.

| Compound Agent | MIC (μg/ml) | Penicillin MIC (μg/ml): | | | | | | | | | | | | % susc.[a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ≤0.008 | 0.015 | 0.03 | 0.06 | 0.12 | 0.25 | 0.5 | 1 | 2 | 4[b] | 8 | 16 | >16 | |
| Ampicillin | ≤0.5 | 16 | 25 | 6 | 6 | 14 | 13 | 10 | 1 | - | - | - | - | - | 34.6[c] |
| | 1 | - | - | - | - | - | - | 2 | 3 | - | - | - | - | - | |
| | 2 | - | - | - | - | - | - | - | 11 | 14 | 3 | - | - | - | |
| | 4 | - | - | - | - | - | - | - | - | 8 | 6 | - | - | - | |
| | >4 | - | - | - | - | - | - | - | - | 1 | 9 | 5 | - | - | |
| Amox/clav | ≤0.12 | 16 | 25 | 6 | 6 | 14 | 9 | 4 | - | - | - | - | - | - | 86.7 |
| | 0.25 | - | - | - | - | - | 3 | 5 | 1 | - | - | - | - | - | |
| | 0.5 | - | - | - | - | - | 1 | 3 | 1 | - | - | - | - | - | |
| | 1 | - | - | - | - | - | - | 1 | 11 | 10 | 3 | - | - | - | |
| | 2 | - | - | - | - | - | - | - | 2 | 5 | 4 | - | - | - | |
| | 4[b] | - | - | - | - | - | - | - | - | 8 | 6 | - | - | - | |
| | 8 | - | - | - | - | - | - | - | - | - | 5 | 1 | - | - | |
| | 16 | - | - | - | - | - | - | - | - | - | - | 4 | - | - | |
| | >16 | - | - | - | - | - | - | - | - | - | - | - | - | - | |
| Cefaclor | ≤0.12 | 1 | - | - | - | - | - | - | - | - | - | - | - | - | 69.9 |
| | 0.25 | 4 | 3 | 1 | - | - | - | - | - | - | - | - | - | - | |
| | 0.5 | 11 | 21 | 3 | 1 | 6 | - | - | - | - | - | - | - | - | |
| | 1 | - | 1 | 2 | 5 | 6 | 4 | 1 | - | - | - | - | - | - | |
| | 2[b] | - | - | - | - | 2 | 3 | 2 | - | - | - | - | - | - | |
| | 4 | - | - | - | - | - | 3 | 1 | - | - | - | - | - | - | |
| | 8 | - | - | - | - | - | 3 | 6 | - | - | - | - | - | - | |
| | 16 | - | - | - | - | - | - | 1 | - | - | - | - | - | - | |
| | >16 | - | - | - | - | - | - | 1 | 15 | 23 | 18 | - | - | - | |
| Cefuroxime | ≤0.2 | 15 | 25 | 4 | 6 | 5 | 1 | 1 | - | - | - | - | - | - | 60.1 |
| | 0.25 | 1 | - | 2 | - | 6 | 6 | 3 | - | - | - | - | - | - | |
| | 0.5 | - | - | - | - | 3 | 2 | 2 | - | - | - | - | - | - | |
| | 1 | - | - | - | - | - | 3 | 3 | - | - | - | - | - | - | |
| | 2[b] | - | - | - | - | - | 1 | 1 | 10 | - | - | - | - | - | |
| | 4 | - | - | - | - | - | - | - | 2 | 5 | 16 | 3 | - | - | |
| | 8 | - | - | - | - | - | - | - | - | 7 | 7 | 10 | - | - | |
| | 16 | - | - | - | - | - | - | - | - | - | - | 3 | - | - | |
| | >16 | - | - | - | - | - | - | - | - | - | 2 | 5 | - | - | |
| Cefotaxime | ≤0.06 | 15 | 25 | 5 | 6 | 10 | 3 | 2 | - | - | - | - | - | - | 69.9 |
| | 0.12 | 1 | - | 1 | - | 4 | 6 | 3 | - | - | - | - | - | - | |
| | 0.25 | - | - | - | - | - | 3 | 4 | 1 | - | - | - | - | - | |
| | 0.5 | - | - | - | - | - | 1 | 1 | 12 | 5 | - | - | - | - | |
| | 1 | - | - | - | - | - | - | 2 | 2 | 18 | 9 | - | - | - | |
| | 2[b] | - | - | - | - | - | - | - | - | - | 6 | - | - | - | |
| | 4 | - | - | - | - | - | - | - | - | - | 2 | - | - | - | |
| | 8 | - | - | - | - | - | - | - | - | - | 1 | 5 | - | - | |
| | >8 | - | - | - | - | - | - | - | - | - | 3 | - | - | - | |
| Ceftriaxone | ≤0.06 | 16 | 25 | 6 | 6 | 12 | 4 | 1 | - | - | - | - | - | - | 60.1 |
| | 0.12 | - | - | - | - | 2 | 4 | 3 | - | - | - | - | - | - | |
| | 0.25 | - | - | - | - | - | 4 | 5 | 1 | - | - | - | - | - | |
| | 0.5 | - | - | - | - | - | 1 | 2 | 12 | 8 | - | - | - | - | |
| | 1 | - | - | - | - | - | - | 1 | 2 | 15 | 12 | - | - | - | |
| | 2[b] | - | - | - | - | - | - | - | - | - | 4 | - | - | - | |
| | 4 | - | - | - | - | - | - | - | - | - | 2 | - | - | - | |
| | 8 | - | - | - | - | - | - | - | - | - | - | 5 | - | - | |
| | >8 | - | - | - | - | - | - | - | - | - | - | - | - | - | |
| Cefepime | ≤0.06 | 16 | 25 | 6 | 6 | 9 | 2 | - | - | - | - | - | - | - | 69.9 |
| | 0.12 | - | - | - | - | 5 | 2 | - | - | - | - | - | - | - | |
| | 0.25 | - | - | - | - | - | 6 | 6 | 1 | - | - | - | - | - | |
| | 0.5 | - | - | - | - | - | 3 | 4 | 11 | 2 | - | - | - | - | |
| | 1 | - | - | - | - | - | - | 2 | 3 | 20 | 9 | - | - | - | |
| | 2[b] | - | - | - | - | - | - | - | - | 1 | 8 | 2 | - | - | |
| | 4 | - | - | - | - | - | - | - | - | - | 1 | 3 | - | - | |
| | 8 | - | - | - | - | - | - | - | - | - | - | - | - | - | |
| | >8 | - | - | - | - | - | - | - | - | - | - | - | - | - | |
| Cephalothin | ≤0.12 | 15 | 22 | - | - | - | - | - | - | - | - | - | - | - | -[d] |
| | 0.25 | 1 | 2 | 3 | 1 | - | - | - | - | - | - | - | - | - | |
| | 0.5 | - | 1 | 3 | 3 | 8 | 1 | 1 | - | - | - | - | - | - | |
| | 1 | - | - | - | 6 | 8 | 2 | - | - | - | - | - | - | - | |
| | 2 | - | - | - | - | 3 | 5 | - | - | - | - | - | - | - | |
| | 4 | - | - | - | - | 1 | 3 | 12 | 2 | - | - | - | - | - | |
| | 8 | - | - | - | - | - | 1 | 2 | 8 | - | - | - | - | - | |
| | 16 | - | - | - | - | - | - | 1 | 13 | 6 | - | - | - | - | |
| | >16 | - | - | - | - | - | - | - | - | 12 | 5 | - | - | - | |
| Cephalexin | ≤0.25 | - | - | - | - | - | - | - | - | - | - | - | - | - | -[d] |
| | 0.5 | - | - | 1 | - | - | - | - | - | - | - | - | - | - | |
| | 1 | 10 | 2 | - | - | - | - | - | - | - | - | - | - | - | |
| | 2 | 5 | 22 | 2 | - | 7 | - | - | - | - | - | - | - | - | |
| | 4 | 1 | 1 | 3 | 5 | 4 | 3 | 2 | - | - | - | - | - | - | |
| | 8 | - | - | - | 1 | 2 | 2 | 1 | - | - | - | - | - | - | |
| | 16 | - | - | - | - | 1 | 6 | 3 | - | - | - | - | - | - | |
| | >16 | - | - | - | - | - | 2 | 6 | 15 | 23 | 18 | 5 | - | - | |
| Cefadroxil | ≤0.25 | 1 | - | 1 | - | - | - | - | - | - | - | - | - | - | -[d] |
| | 0.5 | 7 | 1 | - | - | 2 | - | - | - | - | - | - | - | - | |
| | 1 | 7 | 23 | 3 | 1 | 7 | - | - | - | - | - | - | - | - | |
| | 2 | 1 | 1 | 2 | 4 | 2 | 3 | 2 | - | - | - | - | - | - | |
| | 4 | - | - | 1 | 2 | 3 | 1 | - | - | - | - | - | - | - | |
| | 8 | - | - | - | - | 1 | 5 | 4 | - | - | - | - | - | - | |
| | 16 | - | - | - | - | - | 2 | 2 | 1 | 1 | - | - | - | - | |
| | >16 | - | - | - | - | - | - | 3 | 14 | 22 | 18 | 5 | - | - | |
| Ceftazidime | ≤0.12 | 16 | 16 | - | - | - | - | - | - | - | - | - | - | - | -[d] |
| | 0.25 | - | 9 | 1 | 2 | - | - | - | - | - | - | - | - | - | |
| | 0.5 | - | - | 3 | 4 | 5 | 3 | 1 | - | - | - | - | - | - | |
| | 1 | - | - | 2 | - | 8 | 2 | 1 | - | - | - | - | - | - | |
| | 2 | - | - | - | - | 1 | 3 | 3 | - | - | - | - | - | - | |
| | 4 | - | - | - | - | 4 | 4 | 1 | - | - | - | - | - | - | |
| | 8 | - | - | - | - | - | 1 | 2 | 13 | 14 | 3 | - | - | - | |
| | 16 | - | - | - | - | - | - | 1 | 1 | 9 | 10 | - | - | - | |
| | >16 | - | - | - | - | - | - | - | - | 5 | 5 | 5 | - | - | |

a. CLSI M100-S19 breakpoint criteria were used.
b. Solid vertical and horizontal lines represent susceptible and resistant MIC breakpoint criteria.
c. Based on oral penicillin breakpoint of ≤0.06 μg/ml.
d. No interpretative criteria provided by CLSI.

**Table 3**. Disk diffusion susceptibility breakpoint criteria compared to MIC breakpoints when testing *S. pneumoniae* isolates against five β-lactam antimicrobials.

| Compound Agent | Disk conc. (U or μg) | Susceptibility Criteria | | Intermethod Error rates (%)[a] | | | Acceptable? (% Agreement) | Associated Figure |
|---|---|---|---|---|---|---|---|---|
| | | MIC (μg/ml) | DD zone (mm) | VM | Ma | Mi | | |
| Penicillin | 1 | ≤0.06 | ≥24 | 1.3 | 0.0 | - | Yes (98.7) | 1 |
| | 2 | ≤0.06 | ≥26 | 0.6 | 2.6 | - | Yes (96.8) | - |
| | 10 | ≤0.06 | ≥32 | 0.6 | 2.0 | - | Yes (97.4) | - |
| | 10 | ≤2 | ≥19 | 0.0 | 0.0 | 7.2 | Yes (92.8) | 1 |
| Ceftriaxone | 5 | ≤1 | ≥19 | 0.0 | 0.0 | 3.3 | Yes (96.7) | 2 |
| | 30 | ≤1 | ≥26 | 0.0 | 0.0 | 4.6 | Yes (95.4) | 2 |
| Cefotaxime | 5 | ≤1 | ≥19 | 0.0 | 0.0 | 4.6 | Yes (95.4) | 2 |
| | 30 | ≤1 | ≥27 | 0.0 | 0.0 | 5.2 | Yes (94.8) | - |
| Cefepime | 30 | ≤1 | ≥26 | 0.0 | 0.0 | 7.2 | Yes (92.8) | 2 |
| Amoxicillin/clavulanate | 30 | ≤2 | ≥23 | 0.0 | 0.0 | 3.9 | Yes (96.1) | 2 |

a. VM = very major (false-S), Ma = major (false-R) and Mi = minor.

**Figure 1**. Penicillin MIC versus disk diffusion zone diameter (1- and 10-U disks) for 153 *S. pneumoniae* tested using CLSI methods.

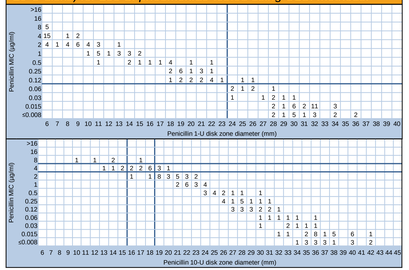
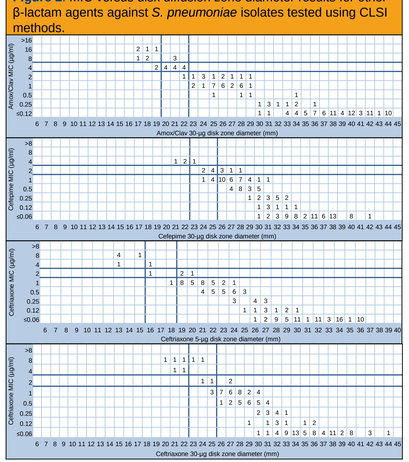



**Figure 2**. MIC versus disk diffusion zone diameter results for other β-lactam agents against *S. pneumoniae* isolates tested using CLSI methods.




## CONCLUSIONS

- The CLSI disk diffusion method to test β-lactam agents against contemporary clinical *S. pneumoniae* and β-haemolytic streptococci isolates can be used against contemporary strains, and the penicillin disk demonstrates acceptable cross-susceptibility predictive accuracy for other selected β-lactam agents.
- The use of lower drug disk concentrations for penicillin (1-U), ceftriaxone (5-μg) and cefotaxime (5-μg) could be used to increase the overall agreement between the broth microdilution and disk diffusion methods. Breakpoints for these DD tests are proposed.
- CLSI should consider re-establishing disk diffusion susceptibility criteria for testing *S. pneumoniae* and β-haemolytic streptococci against some β-lactam agents (penicillins ± enzyme inhibitors, cefepime, cefotaxime and ceftriaxone), as a simple cost effective and accurate method.

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