# Performance of CEM-102 (Fusidic Acid) Susceptibility Testing Reagents; Broth Microdilution, Disk Diffusion and Etest Methods

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## Abstract

**Background**: Fusidic acid (FA) is a steroidal antimicrobial agent with significant potencies against Gram-positive species and acts by preventing bacterial protein synthesis via interacting with elongation factor G. FA has been used in clinical practice for more than four decades as an effective multi-route treatment of skin and skin structure infections (SSSI).

**Methods**: A total of 778 *S. aureus* isolates were collected from USA (561; 53.8% oxacillin-resistant [OXA-R]) and Canadian (217; 46.5% OXA-R) medical centers with nearly equal numbers selected in each of five 2-year increments from 1997 to 2006. Susceptibility (S) testing was performed according to CLSI broth microdilution (BMD; M07-A8) and disk diffusion (DD; M02-A10) methods. The Etest (AB BIODISK, Solna, Sweden) MIC method was tested using manufacturer's package insert instructions.

**Results**: For FA the CLSI BMD MIC method performed well as the reference method. FA was more active against USA *S. aureus* isolates ($MIC_{90}$, 0.12 µg/ml; 100.0% at ≤0.5 µg/ml) compared to Canadian isolates ($MIC_{90}$, 0.25 µg/ml; 93.5% at ≤0.5 µg/ml). BMD results were compared by scattergram analyses to zone diameters around commercially available 5- and 10-µg disks. Excellent correlation (r=0.74-0.76) was observed for both disk contents. Comparing the two disks a r=0.97 correlation was noted. Applying a breakpoint of ≤0.5 µg/ml (S) and ≥2 µg/ml (R) for MIC results and ≥21mm (S) and ≤18mm (R) for DD resulted in 99.9% absolute intermethod categorical agreement with only one minor error. BMD versus Etest MIC results (r=0.77) showed 55.4% identical results and agreement at 99.7% ±1 $log_2$ dilution. A slight trend toward lower MIC results by Etest was observed, 31.2% vs. 13.1% higher.

**Conclusions**: The BMD and DD diagnostic S testing reagents performed at an excellent level of intermethod agreement at 99.7-99.9%. The Etest method was an acceptable alternative to either BMD or DD for FA S testing. FA was very active against contemporary North America staphylococci from SSSI, recognized by these test methods.

## Introduction

*Staphylococcus aureus* is a leading cause of skin and skin structure infections (SSSI), bacterial pneumonia and nosocomial bloodstream infections (BSI). Resistance issues associated with such a virulent and prevalent pathogen have spurred the development of new anti-staphylococcal agents as well as reconsideration of the role of older agents with demonstrated anti-staphylococcal activity.
Fusidic acid (CEM-102) has been suggested to be useful in treating multidrug (MDR)- methicillin (oxacillin)-resistant *S. aureus* (MRSA) and the use of this agent could help to delay the development of resistance to newer potent agents such as linezolid and daptomycin. A promising feature of fusidic acid is the lack of cross resistance with other antimicrobial classes, as a result of the unique mode of action that inhibits bacterial protein synthesis at the translational stage.

Despite the fact that in vitro susceptibility testing of fusidic acid has been performed for many years, fusidic acid is not presently included in the tables of the Clinical and Laboratory Standards Institute (CLSI) and interpretive breakpoints for MIC and disk diffusion testing of fusidic acid against *S. aureus* are not available. In the present study, we provide additional fusidic acid MIC and disk diffusion data and in addition, evaluate the utility of the Etest (AB BIODISK, Solna, Sweden) methodology for testing this agent against a large North American collection of *S. aureus* strains.

## Materials and Methods

Bacterial strains. A total of 728 or 778 non-duplicate clinical isolates of *S. aureus* (52% MRSA) from patients with SSSI or BSI were obtained from more than 30 medical centers in the USA and Canada between 1997 and 2006. All isolates were forwarded to the monitoring laboratory (JMI Laboratories, North Liberty, Iowa, USA) for subsequent identification confirmation and reference antimicrobial susceptibility testing. Identification was performed using an automated system (Vitek; bioMerieux, Hazelwood, Missouri, USA) or conventional manual methods, as required.

Susceptibility testing. All strains were tested by the CLSI broth microdilution method using validated frozen-form panels in cation-adjusted Mueller-Hinton broth. Fusidic acid (also known as CEM-102 [Cempra]) reference powder was obtained from Cempra Pharmaceuticals, Inc. (Chapel Hill, North Carolina, USA). Disk diffusion testing was performed according to CLSI method using Mueller-Hinton agar and two disk concentrations (5- and 10-µg). The zone diameters were measured to the nearest mm using a caliper.

Etest was performed as recommended by the manufacturer (AB BIODISK) using Mueller-Hinton agar, and inoculums of 1-2 X $10^8$ CFU/ml, and incubation at 37°C in air for 18-24 h. The MIC was read at 80% inhibition relative to control growth.

Quality control. Quality control (QC) was performed concurrently with all testing determinations using *S. aureus* ATCC 29213 (MIC) or ATCC 25923 (disks), and *S. pneumoniae* ATCC 49619. The proposed QC ranges for MIC and disk diffusion (10-µg) tests were: 0.06-0.25 µg/ml and 24-32 mm for *S. aureus* ATCC 29213 and ATCC 25923, respectively. The ranges were 4-32 µg/ml and 8-16 mm for *S. pneumoniae* ATCC 49619. Among 61 replicates, all QC values were within control ranges.

Data analysis. Broth microdilution test results were compared by scattergram analysis and regression line equations against zone diameters of inhibition around 5- and 10-µg fusidic acid disks. Interpretive zone size criteria were established using the error-rate-bounded method as described elsewhere. Correlation between the MIC methods (broth microdilution and Etest) were performed by scattergram and regression analysis. Essential agreement (EA) between the two methods was calculated and the percentage of results within ± one $log_2$ dilution step, optimized to 95%.

## Results

- Among 778 strains of *S. aureus* tested in this study, 14 (1.8%) were resistant to fusidic acid as defined by a breakpoint of ≥2 µg/ml (Figure 1a).
- Excellent correlation (r = 0.74) was noted between broth microdilution MIC values and zone diameter results using the 10-µg disk test (Figure 1a).
- For a susceptible MIC breakpoint of ≤0.5 or ≤1 µg/ml, correlate zone diameter breakpoints accurately distinguished susceptible wildtype strains from less susceptible isolates.

Figure 1a. Scattergram comparing fusidic acid (CEM-102) broth microdilution MIC results with zone diameters obtained with a 10-µg fusidic acid disk for 778 isolates of *S. aureus*. The solid lines indicate the interpretive breakpoints proposed in the literature. The broken lines indicate alternative MIC (≤1 µg/ml) and disk diffusion (≥22 mm) interpretive criteria consistent with EUCAST guidelines.

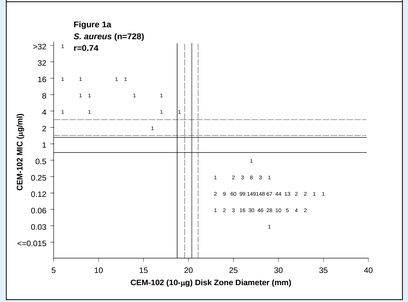
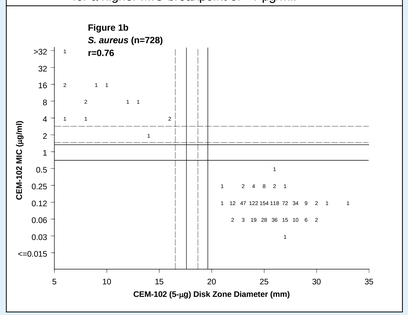



Figure 1b. Scattergram comparing fusidic acid (CEM-102) broth microdilution MIC results with zone diameters obtained with a 5-µg fusidic acid disk for 728 isolates of *S. aureus*. The solid lines indicate the interpretive breakpoints proposed by Skov et al., and broken lines are proposed for a higher MIC breakpoint of ≤1 µg/ml.




- Applying disk zones of ≥21 mm for susceptible (S) and ≤18 mm as resistant (R; see solid vertical and horizontal lines in Figure 1a), the absolute intermethod categorical agreement was 99.9% with only one minor error. A slight adjustment to ≥22 mm (S) and ≤19 mm (R) produced complete (100.0%) intermethod accord.
- Using a susceptible MIC of ≤1 µg/ml and zone diameter criteria of ≥22 mm (S) and ≤19 mm (R) the intermethod agreement was 99.9%.

Figure 2. Scattergram showing the excellent correlation obtained with a 5-µg and 10-µg fusidic acid disk diffusion tests (728 strains).

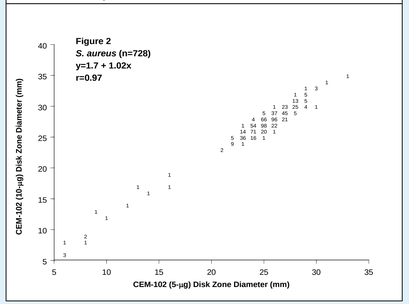
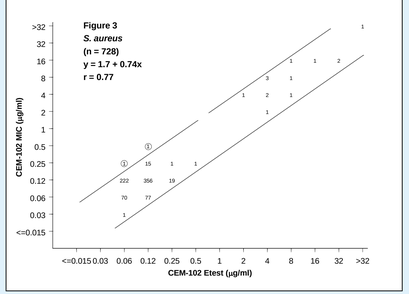



Figure 3. Comparison of fusidic acid broth microdilution and Etest MIC results for 728 isolates of *S. aureus* (r = 0.77).




- Disks containing 5- and 10-µg of fusidic acid demonstrated excellent agreement with reference broth microdilution tests (Figure 1b). Furthermore, an outstanding correlation between the 5- and 10-µg disk zone diameters was observed (r = 0.97; Figure 2).
- The correlation of the fusidic acid reference broth microdilution results with the MIC values produced by Etest showed an essential agreement at 99.7% ± one $log_2$ dilution step with 55.4% identical MIC values (Figure 3).

## Conclusions

- Although both fusidic acid disk concentrations (5- and 10- µg) showed excellent correlation with broth microdilution results, the 10-µg disk is more widely available and/or internationally preferred.
- The Etest proved to be an acceptable alternative method to determine fusidic acid MIC results for *S. aureus* with an intermethod agreement comparable to the CLSI broth microdilution method (e.g. >99%).
- In summary, the in vitro diagnostic tests for fusidic acid (CEM-102) and *S. aureus* performed at a highly acceptable level of intermethod agreement and breakpoints suggested provide harmonization with current EUCAST criteria.

## References

1. Anderson JD (1980). Fusidic acid: new opportunities with an old antibiotic. *Can Med Assoc J* 122: 765-769.
2. Clinical and Laboratory Standards Institute (2009). *M02-A10. Performance standards for antimicrobial disk susceptibility tests; approved standard - tenth edition*. Wayne, PA:CLSI.
3. Clinical and Laboratory Standards Institute (2009). *M07-A8. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard - eighth edition*. Wayne, PA:CLSI.
4. Clinical and Laboratory Standards Institute (2008). *M23-A3. Development of in vitro susceptibility testing criteria and quality control parameters - third edition*. Wayne, PA:CLSI.
5. Clinical and Laboratory Standards Institute (2009). *M100-S19. Performance standards for antimicrobial susceptibility testing. 19th informational supplement*. Wayne, PA:CLSI.
6. Cornaglia G, Rossolini GM (2009). Forthcoming therapeutic perspectives for infections due to multidrug-resistant Gram-positive pathogens. *Clin Microbiol Infect* 15: 218-223.
7. Skov R, Frimodt-Moller, N Espersen F (2001). Correlation of MIC methods and tentative interpretive criteria for disk diffusion susceptibility testing using NCCLS methodology for fusidic acid. *Diagn Microbiol Infect Dis* 40: 111-116.
8. Toma E, Barriault D (1995). Antimicrobial activity of fusidic acid and disk diffusion susceptibility testing criteria for Gram-positive cocci. *J Clin Microbiol* 33: 1712-1715.