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 staphylococcal and staphylococci-like organism agents that targets Gram-positive species and acts by preventing bacterial protein synthesis via interaction with staphylococci-like organism factor G. FA has been used in clinical practice for more than four decades as an effective multicellular element of skin and skin structure infections (SSSI).

Methods: A total of 776 S. aureus isolates were collected from USA (41%), Canada (19%), UK (15%), Mexico (12%), and European (9%) medical institutions and sources from 1997 to 2006. All strains were tested by the disk diffusion (DD) and broth microdilution (BMD; M07-A8) and disk susceptibility (S) testing was performed according to the CLSI broth microdilution (BMD; M07-A8) and disk susceptibility (S) testing was performed according to the manufacturer's package insert instructions. BMD results were compared by scattergram analyses and agreement at 99.7% + agreement with only one minor error. BMD versus disk diffusion resulted in 99.9% absolute intermethod categorical agreement at 2 ng/ml. A correlation was noted. Applying a recognized breakpoint criteria (≥22 mm (S) and ≤19 mm (R) for DD testing) interpreted using CLSI breakpoints (≥21 mm (S) and ≤18 mm (R) for DD testing). Sensitivity and specificity were 89.1% + 98.5% for DD testing and 99.9% + 99.9% for BMD testing. Interpretive breakpoints were 0.06 to 0.5 µg/ml and zone diameters were measured to the nearest millimeter using a caliper. Etest was performed as recommended by the manufacturer (AB BIODISK) using Mueller-Hinton II plates, 0.25- and 1.0 µg/ml disks. The ranges were considered as the results with reference to current CLSI criteria.

Results: For Australia, BMD MIC method performed well as the reference method. FA was active against USA S. aureus isolates (MICs: 0.12 µg/ml); 100.0% at ≤0.5 µg/ml) compared to Canadian (MICs: 0.25 µg/ml; 93.5% at ≤0.5 µg/ml). The ranges were considered as the results with reference to current CLSI and to demonstrate anti-staphylococcal activity. Fusidic acid has been performed for many years, as a result of the unique mode of action that has been demonstrated against a large North American collection of S. aureus isolates.

Materials and Methods

Staphylococcus aureus is a leading cause of skin and skin structure infections (SSSI) in community and nosocomial bloodstream infections (BSI). Resistance issues associated with such a prevalent and virulent organism have been a concern for the development of new anti-staphylococcal agents as well as recognition of the role of other agents with demonstrated anti-staphylococcal activity. Fusidic acid (CEM-102) has been shown to be useful in treating multidrug-resistant (MDR)- methicillin-resistant S. aureus (MRSA) and the use of this agent could help to delay the development of resistance to newer potential 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 CLSI breakpoints (CLSI 2008). Fusidic acid has been shown to have a broad spectrum of activity against a wide variety of Gram-positive pathogens. It is an effective agent against Staphylococcus aureus infections, and has been shown to have excellent activity against this organism. Fusidic acid has been demonstrated to have excellent activity against methicillin-resistant Staphylococcus aureus (MRSA) isolates. The proposed QC ranges for MIC of fusidic acid should be ≤0.12 µg/ml and zone diameters ≥22 mm (S) and ≤19 mm (R). The MIC methods (broth microdilution and Etest) were interpreted using NCCLS methodology for fusidic acid.

References

• Using a susceptible MIC of 0.05 µg/ml and zone diameter breakpoints of 21 mm (S) and 19 mm (R) the interpretive agreement was 99.9%.

Conclusions

• Disks containing 5-10 µg of fusidic acid demonstrated excellent agreement with reference broth microdilution tests (Figure 1a). Using CEM-102 (10 µg) Disk Zone Diameter (mm) Figure 2. Sensitivity and specificity were 89.1% + 98.5% for DD testing and 99.9% + 99.9% for BMD testing. Interpretive breakpoints were 0.06 to 0.5 µg/ml and zone diameters were measured to the nearest millimeter using a caliper.

Figure 1a. Scattergram comparing fusidic acid (CEM-102) broth microdilution and Etest results with the MIC methods for S. aureus ATCC 49619. The proposed QC ranges for MIC of fusidic acid should be ≤0.12 µg/ml and zone diameters ≥22 mm (S) and ≤19 mm (R). The MIC methods (broth microdilution and Etest) were interpreted using NCCLS methodology for fusidic acid.

Figure 1b. Scattergram comparing fusidic acid (CEM-102) broth microdilution results with the MIC methods produced by Eltest and the expected MIC (≤0.015 µg/ml) and zone diameters (<21 mm (S) and ≤18 mm (R) for DD testing) interpreted using CLSI breakpoints (≤0.06 µg/ml). The r value noted was 0.77 (p < 0.01) for the comparison between the two methods. The solid line indicates the interpretive criteria for CEM-102 (10 µg) disk diffusion testing and the dotted line indicates alternative MIC (1 µg/ml) and zone diameter (21 mm) interpretive criteria consistent with EUCAST.

Figure 3. Comparison of fusidic acid broth microdilution and Etest results with the MIC methods for S. aureus isolates of 150 µg/ml and area under the curve (AUC) were performed.

Figure 4. Comparison of fusidic acid broth microdilution and Etest results with the MIC methods for S. aureus isolates of 150 µg/ml and area under the curve (AUC) were performed.

Figure 5. Comparison of fusidic acid broth microdilution and Etest results with the MIC methods for S. aureus isolates of 150 µg/ml and area under the curve (AUC) were performed.

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