P005

Fosfomycin susceptibility testing of *S.aureus* with different commercial methods

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BACKGROUND

Fosfomycin has recently been used for the treatment of serious systemic infections caused by MDR microorganisms. As a common hospital-acquired Gram-positive pathogen, MRSA can cause a wide spectrum of diseases, including bacteremia, pneumonia, osteomyelitis and cellulitis, endocarditis, septic shock and other infectious diseases

Recent studies have demonstrated a good *in vitro* activity of fosfomycin against MRSA clinical isolates.

The clinical use of fosfomycin requires the *in vitro* testing of the drugs in order to be categorized correctly in clinical reports. According to EUCAST, the only approved MIC method for testing fosfomycin susceptibility is the agar dilution (AD) method, using MH agar plates supplemented with 25 mg/L glucose-6-phosphate. However, agar dilution is cumbersome and not routinely performed.

Our aim was to compare an AD commercial test with the reference AD method, and Gradient test (GT) with AD methods for *Staphylococcus aureus* clinical isolates.

MATERIALS AND METHODS

Fosfomycin antimicrobial susceptibility testing was performed on 80 *S.aureus* (n.70 MRSA and n.10 MSSA) clinical isolates (bone, lower respiratory tract and bloodstream infections, and infective endocarditis), according to the gradient test and AD method as described by the Clinical and Laboratory Standards Institute 2014. The gradient test was carried out on Mueller Hinton agar with strips containing fosfomycin and Glucose-6-Phosphate (Liofilchem, Roseto degli Abruzzi, Italy). For susceptibility testing by the AD method, Mueller Hinton agar plates, supplemented with 25 µg/ml glucose-6-phosphate (Sigma Aldrich Co, Italy) were inoculated with 10^4 CFU/ml and incubated in ambient air at 35 °C for 16 to 18 h.

The susceptibility testing by a commercial AD method was performed as recommended by the manufacturer (Liofilchem, Roseto degli Abruzzi, Italy).

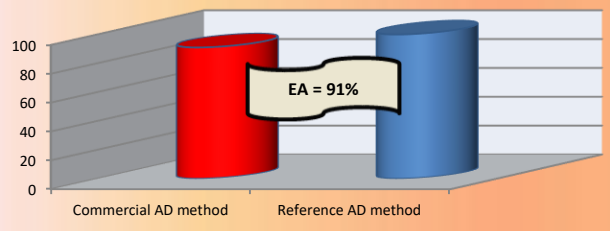
RESULTS

According to the selected breakpoints, fosfomycin, performed by the reference AD method, inhibited 60% of the tested strains (n.49 MRSA and n.9 MSSA). The results of the commercial AD method, compared with those obtained from the AD method, used as reference method, demonstrated a categorical agreement (CA) of 100% and an essential agreement (EA) of 91%. The comparison between GT and reference AD methods showed a CA of 82.5% for all staphylococci tested.

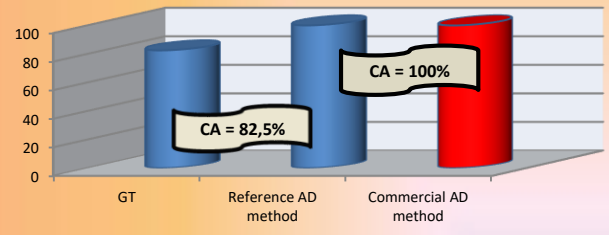
Fosfomycin MIC distribution (mg/L)

Clinical isolates	Methods	≤16	24	32	48	64	>64	% R
S.aureus MRSA n.70	GT	42	5	1	1	4	17	22 (31,4)
	Commercial AD method	21	-	19	-	3	27	30 (42,8)
	AD reference method	22	-	18	-	3	27	30 (42,8)
S.aureus MSSA n.10	GT	8	-	-	-	-	2	2 (20)
	Commercial AD method	7	-	2	-	-	1	1 (10)
	AD reference method	8	-	1	-	-	1	1 (10)

Essential agreement between commercial AD method and reference AD method



Categorical agreement between GT and reference AD methods



CONCLUSIONS

The results obtained comparing the commercial AD method and the reference AD method confirmed the feasibility to characterize fosfomycin susceptibility by Liofilchem panels, while the GT method was not suitable as an alternative to the AD method.

In conclusion, the new commercial method was easy and rapid to use, demonstrating it to be a valid alternative to the reference AD method in routine laboratory practice.