IN-VITRO EFFICACY OF FOSFOMYCIN AGAINST COMMONLY ISOLATED BACTERIA

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SYNOPSIS

Fosfomycin, a new antibiotic not related to any antibiotic in use is a small molecule with a simple structure. The *in vitro* efficacy of this drug against commonly isolated gram positive and gram negative bacteria was studied. Results showed that organisms such as *Staph. aureus*, both methicillin sensitive and resistant, *H. influenzae*, ø-haemolytic streptococci, *S. typhi*, *S. marcescens*, Proteus species, and *Ps. aeruginosa* were sensitive to fosfomycin. Those exhibiting resistance were *F. meningosepticum*, Flavobacterium species and Klebsiella species. A third category included *E. coli*, *Strep. faecalis* and Shigella species which showed intermediate susceptibility. It is possible that fosfomycin may be useful against infections caused by methicillin-resistent *Staph. aureus* and multiply-resistant *Ps. aeruginosa* but clinical trials are first necessary to substantiate this.

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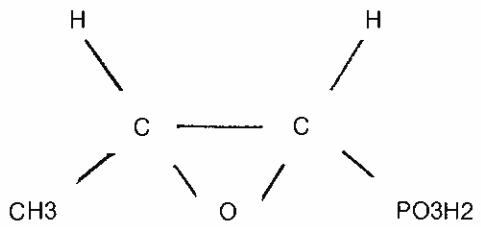
INTRODUCTION

Fosfomycin, discovered in 1967, is an entirely new antibiotic not related to any antibiotics in current use. This broad-spectrum antibiotic is chemically a (−) *cis*-1, 2-epoxypropl phosphonic acid and it was originally isolated in Spain from cultures of Streptomyces species. It is a small molecule (molecular weight 138) with a simple structure and is now chemically synthesized (1). In the very early publications, the generic name of phosphonomycin was used (2).



This antibiotic acts by interfering with the initial stages of bacterial cell-wall synthesis unlike the B-lactam antibiotics which act on the final stages of cell-wall synthesis. It is bactericidal and broad-spectrum being active against both gram positive and gram negative bacteria and against some pseudomonads but not active against Bacteroides species. Owing to its unique chemical structure and mode of action it does not exhibit cross-resistance with clinically utilized antibiotics. Multi-resistant bacteria isolated in hospital laboratories from human infections were found to be sensitive to fosfomycin (1).

This drug, with its wide spectrum activity and high rate of diffusion into tissues and body fluid has been in use in various parts of Europe for almost two decades (3,4) but has only been recently introduced to Malaysia.

The present study was undertaken to determine the *in vitro* antibacterial efficacy of fosfomycin against commonly isolated bacteria, both gram positive and gram negative, from infections arising in the inpatient as well as the outpatient setting of the University Hospital, Kuala Lumpur.

MATERIALS AND METHODS

The bacterial strains used were fresh clinical isolates from specimens of wound swabs, cerebrospinal fluid, sputum, genital swabs, blood cultures, urine and stools, submitted to the bacterial diagnostic laboratories of the University Hospital. These strains were identified by biochemical tests according to the methods described by Cowan and Steel (5).

Susceptibility testing for antimicrobials other than fosfomycin was carried out by the disc diffusion method using isosensitest agar (Oxoid). The Oxford staphylococcus NCTC 6571, *Escherichia coli* NCTC 10418 and *Pseudomonas aeruginosa* NCTC 10662 were used as controls.

Fosfomycin sensitivity testing was carried out according to the manufacturer's instructions using Mueller-Hinton agar (Oxoid CM 337) and fosfomycin sodium 200 μg discs (Showa Yakuhin Kako Co. Ltd. Japan). The inoculum was prepared by suspending one to two colonies of the organism in 1 ml of sterile nutrient broth. A loopful of this suspension was then seeded onto each agar plate.

Fastidious organisms like ø-haemolytic streptococci and *Haemophilus influenzae* were first grown on solid media. Sufficient growth was then removed and suspended in nutrient broth to give just visible turbidity. Mueller-Hinton agar containing 5% ox blood and chocolate agar made up with Mueller-Hinton agar as base were used for testing ø-haemolytic streptococci and H. *influenzae* respectively.

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TABLE 1
FOSFOMYCIN SUSCEPTIBILITY OF BACTERIA

Micro-organisms	No. Tested	Susceptibility to Fosfomycin No. (%) of strains S	I	R
Staph. aureus				
methicillin-sensitive	20	18(100)	1(5)	1(5)
methicillin-resistant	20	20(100)	0(0)	0(0)
β-haem. streptococci	20	18(90)	2(10)	0(0)
Strep. faecalis	20	2(10)	13(65)	5(25)
H. influenzae	20	20(100)	0(0)	0(0)
S. typhi	20	18(90)	2(10)	0(0)
Salmonella sp.	20	11(55)	7(35)	2(10)
Shigella sp.	19	3(15.8)	12(63.2)	4(21)
E. coli	20	4(20)	14(70)	2(10)
Proteus sp	20	16(80)	3(15)	1(5)
Klebsiella sp.	20	0(0)	6(30)	14(70)
Serratia marcescens	20	18(90)	1(5)	1(5)
Acinetobacter sp.	20	2(10)	15(75)	3(15)
Enterobacter sp.	20	6(30)	7(35)	7(35)
Ps. aeruginosa	20	9(45)	10(50)	1(5)
F. meningosepticum	20	1(5)	6(30)	13(65)
Flavobacterium sp.	17	0(0)	0(0)	17(100)

S — very sensitive with zone diameter ⩾ 27mm
I — fairly sensitive with zone diameter 17 – 26 mm
R — resistant with zone diameter ⩽ 16 mm

The plates were incubated overnight at 35 – 37°C and diameters of inhibition zones were recorded. The interpretation of fosfomycin sensitivity was according to the information supplied by the manufacturer of Fosfomycin Showa Disk.

RESULTS

A total of 329 organisms were tested comprising both gram positive and gram negative facultative anaerobes. The strains were classified as very sensitive(S) if the zone diameter was ⩾ 27mm; fairly sensitive (I) when the zone diameter was 17–26mm and resistant (R) if the zone diameter was ⩽ 16mm.

The results of the sensitivity tests are shown in Table 1 and accordingly the organisms seemed to fall into four groups; The first group comprised organisms which were mostly susceptible (⩾ 80%) to fosfomycin. These were methicillin resistant *Staphylococcus aureus* (MRSA), methicillin sensitive staphylococci, *H. influenzae*, ø-haemolytic streptococci including *Streptococcus pyogenes* and *group B streptococci*, *Salmonella typhi*, *Serratia marcescens* and Proteus species, both indole positive and indole negative strains.

The second group consisted of those organisms that were mostly resistant to fosfomycin (⩾ 80%), such as *Flavobacterium meningosepticum*, Flavobacterium species and Klebsiella species.

Organisms of intermediate susceptibility made up the third group and these were Acinetobacter species, *Escherichia coli*, *Streptococcus faecalis* and Shigella species.

The fourth group was made up of organisms that were either very sensitive or of intermediate sensitivity. These were *Pseudomonas aeruginosa*, Salmonella species and Enterobacter species.

DISCUSSION

New antibiotics are constantly being discovered and marketed vigorously by drug companies. Before introducing these for clinical application it is useful to assess their *in vitro* efficacy against both commonly isolated pathogens as well as against important pathogens in our community. We have chosen to do this for fosfomycin by the simple disc diffusion technique. The interpretation of sensitivity or resistance is based on information supplied by the manufacturers of Fosfomycin Showa Disk who have correlated the inhibition zone diameters with clinically relevant MIC values.

Our results are quite similar to those obtained by investigators from other parts of the world. Goto (6) found most strains of *Staph. aureus*, Salmonellae and *Pseudomonas aeruginosa* to be sensitive. Ullmann and Lindemann (7) tested fosfomycin against *Ps. aeruginosa* and *Serratia marcescens* and found 86.4% of the former and 91% of the latter to be sensitive.

It would appear from our results that fosfomycin would be very useful against our local strains of *Staph. aureus* especially our methicillin-resistant strains which tend to exhibit multiple resistance to commonly used antibiotics. Of the 20 methicillin-resistant *Staph. aureus* that we tested, 17 were also tested against gentamicin, fusidic acid, rifampicin and vancomycin. Among these, 16 strains were found to be resistant to gentamicin, one each resistant to fusidic acid and rifampicin and none resistant to vancomycin.

Clinically, in cases of infections with methicillin-sensitive Staphylococci, *H. influenzae* or ø-haemolytic streptococci, fosfomycin can be a valuable alternative drug for patients with a history of penicillin or ampicillin allergy. As regards gram negative bacteria, fosfomycin may be useful against infections caused by *Serratia marcescens*, Salmonellae, *Ps. aeruginosa* and Proteus species. Most of the Serratia strains isolated in the University Hospital, Kuala Lumpur are resistant to five or more antibiotics and 8% of them are resistant to gentamicin (8). Of the *Ps. aeruginosa* tested in this study 25% were found to be resistant to gentamicin. Hence fosfomycin appears to be as good as gentamicin against *Serratia marcescens* and more useful than gentamicin against *Ps. aeruginosa*. Besides being active against more Pseudomonas strains, fosfomycin is free from the toxicity that limits aminoglycoside therapy (3). The high sensitivity of these strains to fosfomycin may be due to the fact that this drug has only been very recently introduced into Malaysia.

In conclusion it is important to keep in mind that these are only *in vitro* results and extrapolation to the clinical setting can be valid only if clinical trials with fosfomycin substantiate the laboratory findings.

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