Susceptibility Study of Common Bacterial Isolates in Periprosthetic Joint Infection to Manually-mixed Fosfomycin Compared with Gentamicin Pre-mixed PMMA Bone Cement

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Objective: *This study aimed to compare in vitro susceptibility of S. aureus and S. epidermidis, which are common isolates in periprosthetic joint infection, to manually-mixed fosfomycin with, the more commonly used, gentamicin pre-mixed bone cement.*

Materials and Methods: *Modified disk diffusion technique was performed. Two clinical and 1 laboratory strains of S. aureus and 3 laboratory strains of S. epidermidis were cultured on agar plates. Manually-mixed fosfomycin (4 g fosfomycin in 40 g PMMA) and gentamicin pre-mixed (0.5 g gentamicin in 40.8 g PMMA) bone cement were prepared by using standard sterile mixing technique to produce 6 mm- spherical cement beads. The susceptibility of all bacteria on agar plates were tested against these cement beads. After incubation, the zone of inhibition was measured.*

Results: *Disk diffusion susceptibility test demonstrated that all 3 strains of standardized S. aureus and S. epidermidis are more susceptible to manually-mixed fosfomycin cement beads than gentamicin pre-mixed beads as shown by the wider inhibition zone of each cement bead on agar plate. The mean difference of inhibition zone of both types of cement beads for S. aureus strain 1, 2 and 3 are 4.65 (3.24, 6.06), 17.33 (16.71, 17.94) and 9.44 (8.63, 10.25), for S. epidermidis strain 1, 2 and 3 are 12.39 (11.74, 13.03), 12.95 (12.93, 12.97) and 11.62 (11.26, 11.98), which are all statistically significant ($p<0.01$).*

Conclusions: *The susceptibility of S. aureus and S. epidermidis to manually-mixed fosfomycin cement beads (4 g of fosfomycin in 40 g PMMA) are significantly more than to pre-mixed gentamicin beads (0.5 g gentamicin in 40.8 g PMMA) as demonstrated by in vitro disk diffusion study.*

Keywords: *Susceptibility study, fosfomycin, gentamicin, antibiotic bone cement, periprosthetic joint infection*

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Introduction

Periprosthetic joint infection (PJI) is a serious complication following total joint arthroplasty[(1)] and is the most common reason for revision total knee arthroplasty (TKA)[(2)] and the second most common reason for revision total hip arthroplasty (THA)[(3,4)]. *Staphylococcus aureus* and *Staphylococcus epidermidis* are organisms most commonly isolated from the infection site associated with hip and knee arthroplasty[(5-7)]. Although there are various options for treatment of PJI, a two-stage revision arthroplasty including removal of the prosthesis and cement, thorough debridement, placement of an antibiotic-loaded PMMA cement spacer, a course of intravenous antibiotics, and a delayed second-stage revision arthroplasty is considered to be a current standard of care, especially for late and chronic infection[(8,9)].

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Antibiotic PMMA bone cement commonly used to prepare spacer are usually pre-loaded with aminoglycosides. Gentamicin is the most commonly chosen antibiotic because of its broad antimicrobial spectrum, heat stability[(10)], high water solubility and low allergenicity[(11)]. However, with the frequent and increasing local use of gentamicin-loaded PMMA bone cement, gentamicin-resistant staphylococci are increasingly prevalent[(12)]. The resistant rates of gentamicin have been reported ranging from 41% to 74% [(13-15)]. In a study of 93 staphylococci from patients with PJI, 41% and 66% of the isolates were resistant to gentamicin and tobramycin, respectively[(13)]. Thornes et al[(16)] also demonstrated in an animal study the development of gentamicin resistance by initially gentamicin-susceptible *S. epidermidis* strains after exposure to gentamicin-loaded PMMA. The increasing number of these reports has inevitably led to the use of new antimicrobial agents for incorporation into PMMA bone cement.

Fosfomycin[(17)] is a bactericidal antibiotic that interferes with cell wall synthesis in both

Gram-positive and Gram-negative bacteria by inhibiting the initial step involving phosphoenolpyruvate synthase. It has broad and excellent antimicrobial activity against Gram-positive cocci, such as methicillin-sensitive and methicillin-resistant *S. aureus (MSSA and MRSA)*[18], cephalosporin- and penicillin-resistant *Streptococcus pneumoniae*[19,20], and *Enterococcus species*, even in vancomycin-resistant strains[21]. In vitro study, most Gram-positive cocci are more susceptible to fosfomycin than gentamicin[18]. Fosfomycin-loaded PMMA may be a better choice of local antibiotic delivery system in managing PJI. Review of the literature reveals no previous report on the susceptibility study of common isolates in PJI to fosfomycin PMMA cement.

This in vitro study aimed to compare the susceptibility of *S. aureus* and *S. epidermidis* to manually-mixed fosfomycin and gentamicin pre-mixed PMMA bone cement.

Materials and Methods

The high viscosity PMMA cement used for manually-mixed with fosfomycin was Palacos® R *(Heraeus GmbH, Germany)(1 pouch of 40.0 g powder contains: poly (methyl acrylate, methyl methacrylate) 33.8 g zirconium dioxide 5.9 g hydrous benzoyl peroxide 0.3 g and 1 ampoule of 20 ml liquid contains: methyl methacrylate 18.4 g N,N-dimethyl-p-toluidine 0.4 g)* and the gentamicin pre-mixed cement was Palacos® R+G *(Heraeus GmbH, Germany)(1 pouch of 40.8 g powder contains: poly (methyl acrylate, methyl methacrylate) 33.6 g zirconium dioxide 6.1 g hydrous benzoyl peroxide 0.3 g gentamicin base (as sulphate) 0.50 g, and 1 ampoule of 20 ml liquid contains: methyl methacrylate 18.4 g N,N-dimethyl-p-toluidine 0.4 g).* Mixing of the cement was performed without vacuum. Four grams powder of fosfomycin *(Fosmicin®, Thai Meiji Pharmaceutical, Thailand)* was admixed with 40 grams of Palacos® R powder before adding into 20 ml liquid in a sterile mixing device and carefully stirred with a sterile mixing rod until a homogeneous mass is obtained. Gentamicin pre-mixed cement was prepared in the same manner. The liquid cement was poured into 2 halves of a silastic cement block to produce 6 mm spherical beads.

Common bacterial isolates in periprosthetic joint infection were prepared as followed: 2 strains of *S. aureus* were isolated from periprosthetic joint infection specimens in the study hospital, another strain was standard laboratory isolate. All 3 strains of *S. epidermidis* were laboratory strain.

Susceptibility study was performed *in vitro* by using a modification of the standard disk diffusion technique[22,23]. An overnight culture of bacteria was diluted in phosphate buffered saline (pH 7.4) yielding about 2 x 10^7 colony-forming units per milliliter. Aliquots (0.1 ml) were pipetted on to the dried Mueller-Hinton Agar plates and inoculated by streaking the entire surface in three directions. After each streak, the plate was rotated 60 degrees to obtain an even distribution of the inoculum. Three plates of each bacterial strain, for fosfomycin manually-mixed cement bead, gentamicin pre-mixed cement bead and for antibiotic control disk (total 18 plates), were prepared and labelled (Figure 1). All plates were left dry for no more than 15 minutes. Four antibiotic cement beads were placed at a distance 30 mm apart on each plate. Flame-sterilized forceps was used to gently press each bead onto the agar to ensure that the bead was attached to the agar. Plates were incubated overnight at an incubation temperature of 37 °C (98.6 °F). After incubation, the agar plates were examined at 24 hours. Zone of inhibition was measured (Figure 2) using sliding caliper Vernier.

Analysis was undertaken on the inhibition zone defined as the mean (X_1+X_2/2) inhibition zone diameter subtracted the diameter of the cement bead (Y) (Figure 2). Comparison between the inhibition zones of both antibiotic cement beads in each strain of bacteria was performed. The data had normal distribution according to the standard deviation, therefore an independent t-test was used to analyze. The mean difference in inhibition zone was considered statistically significant if $p < 0.05$.

Results

Disk diffusion test demonstrated that mean inhibition zone (of 4 beads on each plate) of manually-mixed fosfomycin cement beads exhibited to *S. aureus* strain 1, 2 and 3 are 14.9, 41.07 and 31.48 mm compared to 10.25, 23.74 and 22.04 mm of pre-mixed gentamicin cement beads (Table 1). Mean difference and *95% CI* are 4.65*(3.24, 6.06)*, 17.33*(16.71,17.94)* and 9.44*(8.63, 10.25)* which are statistically significant *($p < 0.01$).*

Mean inhibition zone of manually-mixed fosfomycin cement beads exhibited to *S. epidermidis* strain 1, 2 and 3 are 33.14, 36.93 and 30.99 mm compared to 20.75, 23.99 and 19.37 mm of pre-mixed gentamicin cement beads (Table 2). Mean difference and *95%CI* are 12.39*(11.74, 13.03)*, 12.95*(12.93,12.97)* and 11.62*(11.26,11.98)* which are statistically significant *($p < 0.01$).*

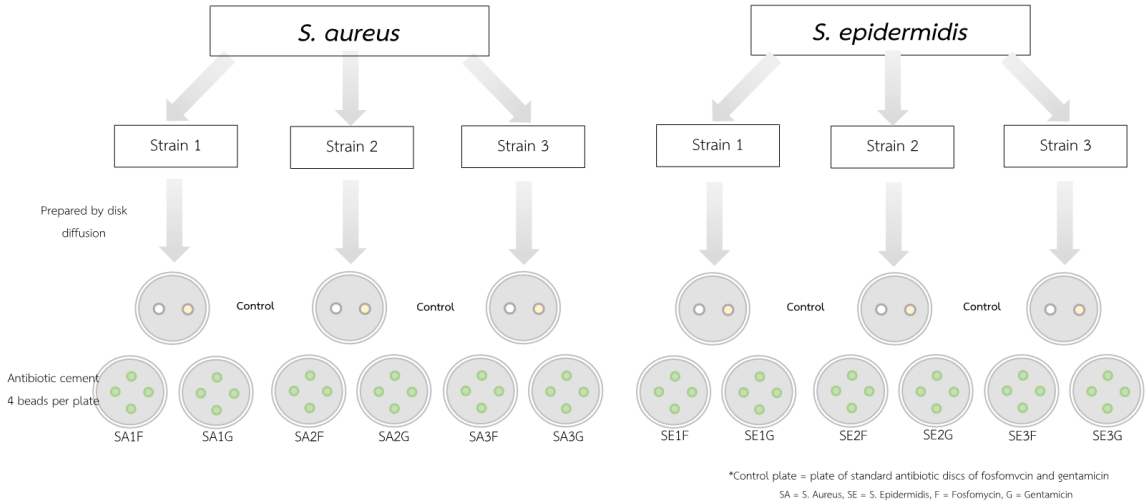

Fig.1 The preparation of agar plate for susceptibility study of *S. aureus* and *S. epidemidis* by disk diffusion technique.

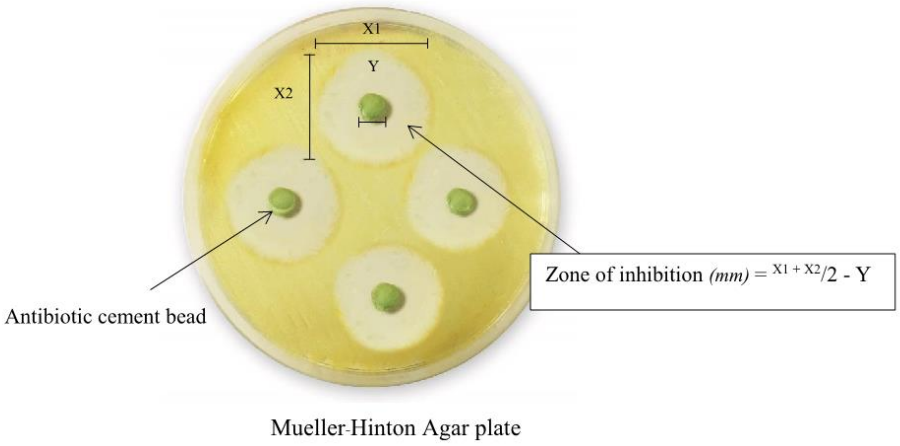

Fig.2 Inhibition effect of antibiotic cement beads as demonstrated by measuring zone of inhibition.

Table 1 Comparison of inhibition effect of manually-mixed fosfomycin and pre-mixed gentamicin cement beads on *Staphylococcus aureus*

Staphylococcus aureus	**Antibiotic cement bead**	**Inhibition zone in 24 hr. *(mean) (mm)***	**Mean difference *(95% CI)***	***p-value***
Strain 1	*manually-mixed* fosfomycin	14.90	4.65 *(3.24, 6.06)*	*<0.01*
	pre-mixed gentamicin	10.25		
Strain 2	*manually-mixed* fosfomycin	41.07	17.33 *(16.71, 17.94)*	*<0.01*
	pre-mixed gentamicin	23.74		
Strain 3	*manually-mixed* fosfomycin	31.48	9.44 *(8.63, 10.25)*	*<0.01*
	pre-mixed gentamicin	22.04		

Table 2 Comparison of inhibition effect of manually-mixed fosfomycin and pre-mixed gentamicin cement beads on *Staphylococcus epidermidis*

Staphylococcus epidermidis	**Antibiotic cement bead**	**Inhibition zone in 24 hr. *(mean) (mm)***	**Mean difference *(95% CI)***	***p-value***
Strain 1	*manually-mixed* fosfomycin	33.14	12.39 *(11.74, 13.03)*	*<0.01*
	pre-mixed gentamicin	20.75		
Strain 2	*manually-mixed* fosfomycin	36.93	12.95 *(12.93, 12.97)*	*<0.01*
	pre-mixed gentamicin	23.99		
Strain 3	*manually-mixed* fosfomycin	30.99	11.62 *(11.26, 11.98)*	*<0.01*
	pre-mixed gentamicin	19.37		

Discussion

Periprosthetic joint infection (PJI) is becoming the leading cause of failure after primary and revision total knee and total hip arthroplasty[24,25]. Periprosthetic infections associated with arthroplasty are predominantly caused by bacteria able to form biofilms on implant surface[26]. *S. aureus and S. epidermidis* are respectively at the first and second positions of the list of the leading bacterial isolates, followed by a certain number of coagulase-negative staphylococci (CoNS) species, such as *S. hominis, S. haemolyticus, S. capitis, and S. warneri*[27,28]. Systemic antibiotics, which are commonly used to treat PJI, are not sufficiently effective to eradicate deep infections because of the impaired blood circulation and low antibiotic concentration at the implantation site[29]. Use of antibiotic-loaded PMMA cement spacers is considered a standard of care for two-stage revision arthroplasty. These spacers provide direct local delivery of antibiotics while preserving patient mobility and facilitate re-implantation surgery[30]. Antibiotic cement spacer deliver high doses of antibiotics at the site of the infection and can provide local concentration higher than those achieved with systemic antibiotics alone, with minimal effect on serum or urine levels[31,32].

The type of antibiotics used to mix into the cement spacer varies, with aminoglycosides and vancomycin most commonly used[33]. For PJI requiring two-stage revision arthroplasty, the selection of antibiotic that most of the causative organisms are susceptible to is important factor for the clinical efficacy of the antibiotic cement spacer. Although gentamicin in bone cement was reported to be a potentially more effective for infection prophylaxis in primary total joint arthroplasty[34] by providing a broad antibacterial spectrum against *methicillin-sensitive S. aureus, CoNS, Pseudomonas aeruginosa* and *Escherichia coli* as compared with the bone cement loaded with vancomycin, teicoplanin, ceftazidime, imipenem, piperacillin or tobramycin, but for cases with PJI, aminoglycoside resistance seems to be increasing and potentially impact its utility in cement spacer[3,13,35,36], necessitating the use of other antibiotics or combination of antibiotics in bone cement to enable more effective infection elimination.

Fosfomycin, originally named phosphonomycin, was discovered in Spain in 1969[37], it is a phosphonic acid derivative, with an extremely low molecular weight, and shows almost no binding to proteins[38]. This unique antibiotic is chemically unrelated to any other known antibacterial agent. It has a unique mechanism of action that may provide a synergistic effect to other antibiotics, including beta-lactams, aminoglycosides, and fluoroquinolones. Intravenous fosfomycin has been administered in combination with other antibiotics for the treatment of nosocomial infections due to multidrug-resistant Gram-positive and Gram-negative bacteria[38]. Biofilms forming on prosthesis surfaces usually play significant role in the development and persistence of several periprosthetic infections. The chronicity of infection is also by the antibiotic resistance bacteria in the biofilms and the stability of the biofilms. Fosfomycin has shown antimicrobial activity against biofilms and can break up biofilms to enhance the permeability of antibiotics, particularly in combination with fluoroquinolones or aminoglycosides[39-41].

To compare the susceptibility of common isolates in PJI, which are *S. aureus* and *S. epidermidis*, to local cement spacer of fosfomycin and gentamicin, this in vitro study used modification of standard disk diffusion technique which is a standard method for susceptibility testing of causative organisms[22,23]. The difference in surface area-to-volume ratio of bone cement was found to have no difference in elution characteristic of antibiotic-loaded cement[42], therefore cement beads were used instead of the cement spacer. Both pre-mixed and manually-mixed antibiotic loaded bone cement also showed no differences in homogeneity and elution characteristics by a

modified disk diffusion technique[43], and although this technique is semi-quantitative method, the inhibition effect has been reported to correlate directly with the local concentration of antibiotic[44,45]. The different amount of fosfomycin and gentamicin mixed in PMMA cement is due to the availability of gentamicin pre-mixed cement (Palacos® R+G, which contains only 0.5 g gentamicin), whereas 10 percent of antibiotic is general recommendation for manually-mixed antibiotic PMMA cement used in orthopedic practices. This study demonstrated the more susceptible of all 3 strains of *S. aureus* and also all 3 strains of *S. epidermidis* to fosfomycin cement beads than to gentamicin cement beads as shown by the statistically significant of mean difference of inhibition zone. This makes fosfomycin an interesting antibiotic of choice to mix with PMMA cement and use as local cement spacer in two-stage revision arthroplasty after PJI, especially cases which gentamicin- resistant organisms are suspected.

Although *S. aureus* and *S. epidermidis* are common isolates from PJI, other organisms including variety of Gram-positive and Gram-negative organisms can also be causative organism. This study has the limitation of not including the less common isolates in the susceptibility study.

Conclusion

S. aureus and *S. epidermidis* which are common isolates from periprosthethic joint infection are more susceptible to manually-mixed fosfomycin cement beads (4 g of fosfomycin in 40 g PMMA) than pre-mixed gentamicin cement beads (0.5 g gentamicin in 40.8 g PMMA) as demonstrated by in vitro disk diffusion study.

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Potential conflicts of interest

None

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การศึกษาความไวของเชื้อแบคทีเรียที่แยกได้บ่อยจากการติดเชื้อรอบข้อเทียมต่อซีเมนต์กระดูกที่ผสมฟอสโฟมัยซินด้วยมือเปรียบเทียบกับที่ผสมเจนตามัยซินสำเร็จรูป

ทรงไทย มูลวงศ์,พบ, สุกิจ แสงนิพันธ์กูล, พบ, สุรศักดิ์ วงศ์รัตนชีวิน, ปรด

วัตถุประสงค์: การศึกษาในห้องปฏิบัติการเพื่อเปรียบเทียบความไวของเชื้อ *S. aureus* และ *S. epidermidis* ซึ่งเป็นเชื้อที่เพาะแยกได้บ่อยในการติดเชื้อรอบข้อเทียม ต่อซีเมนต์กระดูกผสมฟอสโฟมัยซิน ด้วยมือกับซีเมนต์ผสมเจนตามัยซินสำเร็จรูป ซึ่งมีการใช้บ่อยกว่า

วัสดุและวิธีการ: ใช้เทคนิค modified disk diffusion โดยเพาะเชื้อ *S. aureus* ที่ได้จากทางคลินิกสองสายพันธุ์ จากห้องปฏิบัติการหนึ่งสายพันธุ์ เพาะเชื้อ *S. epidermidis* จากห้องปฏิบัติการ สามสายพันธ์บนจานวุ้น เตรียมเม็ดซีเมนต์ทรงกลมเส้นผ่าศูนย์กลาง 6 มม. โดยใช้ PMMA 40 กรัมผสม ฟอสโฟมัยซิน 4 กรัม และ PMMA ผสมเจนตามัยซินสำเร็จรูป (PMMA 40.8 กรัม ผสมเจนตามัยซิน 0.5 กรัม) ด้วยเทคนิคปลอดเชื้อมาตรฐาน วางเม็ดซีเมนต์ที่เตรียมนี้บนจานเพาะเชื้อเพื่อศึกษาความไวของเชื้อต่อยาแต่ละชนิดด้วยการวัด zone of inhibition หลังการอบในตู้อบ

ผลการศึกษา: การทดสอบความไวของเชื้อด้วยวิธี disk diffusion พบว่าทั้งสามสายพันธุ์ของ *S. aureus* และ *S. epidermidis* มีความไวต่อเม็ดซีเมนต์ผสมฟอสโฟมัยซินด้วยมือ มากกว่าที่ผสมเจนตามัยซินสำเร็จรูป อย่างมีนัยสำคัญทางสถิติ ($p<0.01$) โดยมี zone of inhibition ที่กว้างกว่า มีค่าเฉลี่ยความแตกต่าง 4.65 (*3.24, 6.06*), 17.33 (*16.71, 17.94*) และ 9.44 (*8.63, 10.25*) สำหรับ *S.aureus* และ 12.39 (*11.74, 13.03*), 12.95 (*12.93, 12.97*) และ 11.62 (*11.26, 11.98*) สำหรับ *S. epidermidis* สายพันธุ์ 1, 2 และ 3 ตามลำดับ

สรุป: การศึกษาด้วยวิธี modified disk diffusion พบว่า *S. aureus* และ *S. epidermidis* ซึ่งเป็นเชื้อที่เพาะแยกได้บ่อยในการติดเชื้อรอบข้อเทียม มีความไวต่อซีเมนต์ผสมฟอสโฟมัยซินด้วยมือ มากกว่าที่ผสมเจนตามัยซินสำเร็จรูป อย่างมีนัยสำคัญทางสถิติ