Effect of Trimethoprim/Sulfamethoxazole (SXT) Disc Distance on Cell Length of SXT-resistant *Escherichia coli*

ผลของระยะห่างจากแผ่นยา Trimethoprim/Sulfamethoxazole (SXT) ต่อความยาวเซลล์ของ *Escherichia coli* ที่ดื้อต่อยา SXT

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ABSTRACT

Most of *Escherichia coli* isolates from stone matrices and urine were resistant to trimethoprim/ sulfamethoxazole (SXT) which may have some adaptations for survivor after expose to SXT. The present study aimed to evaluate the effect of SXT disc distance on cell length of SXT-resistant *E. coli* (SREC). Seven SREC samples were isolated from stone matrices and urine. We performed a systemic evaluation of serially SXT disc distant changes at 0, 5, 10 and 15 mm on bacterial cell length. The data indicated that SXT concentration had the effect on cell length of SREC isolates. There was a negative correlation between bacterial cell length and SXT disc distance ($r = -0.958$, $p < 0.05$). The present study reveals that SXT had the effect on cell separation of SREC isolates. Our study not only provided the description data, but also implied the adaptation of the SREC cell length in SXT condition.

บทคัดย่อ

เชื้อ *Escherichia coli* ส่วนใหญ่ที่แยกได้จากก้อนนิ่ว และปัสสาวะ มักจะดื้อยา trimethoprim/sulfamethoxazole (SXT) โดยเชื้อเหล่านี้อาจสร้างปัจจัยบางอย่างที่เกี่ยวข้องกับการปรับตัว เพื่อให้มีชีวิตอยู่รอดในภาวะที่มียา SXT การศึกษานี้มีวัตถุประสงค์ เพื่อประเมินผลของระยะห่างจากแผ่นยา SXT ต่อความยาวเซลล์ของ *E. coli* ที่ดื้อต่อยา SXT โดยทำการศึกษาใน *E. coli* ที่ดื้อต่อยา SXT จำนวน 7 ตัวอย่าง ที่แยกได้จากก้อนนิ่วและปัสสาวะ คณะผู้วิจัยได้ทำการประเมินผลของระยะห่างจากขอบของแผ่นยา SXT ณ 0, 5, 10 และ 15 มม. ต่อความยาวของเซลล์แบคทีเรียที่เปลี่ยนแปลงไปอย่างเป็นระบบ จากการศึกษาครั้งนี้พบว่าความเข้มข้นของยา SXT มีผลต่อความยาวเซลล์ของ *E. coli* ที่ดื้อต่อยา SXT ซึ่งระยะห่างจากแผ่นยา SXT มีความสัมพันธ์เป็นไปในทิศทางตรงกันข้ามกับความยาวเซลล์ของแบคทีเรียอย่างมีนัยสำคัญทางสถิติ ($r = -0.958$, $p < 0.05$) การศึกษานี้แสดงให้เห็นว่ายา SXT มีผลต่อการแบ่งเซลล์ของ *E. coli* ที่ดื้อต่อยา SXT นอกจากให้ข้อมูลเชิงพรรณนาแล้ว ยังแสดงให้เห็นถึงการปรับตัวของความยาวเซลล์ของ *E. coli* ที่ดื้อต่อยา SXT ในภาวะที่มียา SXT อีกด้วย

Key Words: Trimethoprim/Sulfamethoxazole, *Escherichia coli,* Cell length

คำสำคัญ: ยาไตรเมโทพริม/ซัลฟาเมทโทซาโซล เชื้อเอสเชอริเชีย โคไล ความยาวของเซลล์แบคทีเรีย

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Introduction

Escherichia coli is the most prevalent pathogen which isolated from UTI (Burman et al., 2003) patients and kidney stone patients with bacterial isolates (Tavichakorntrakool et al., 2012). Most of *E. coli* isolates are multi-drug resistance (Chomarat, 2000; Karlowsky et al., 2002; Kiffer et al., 2007; Yengkokpam et al., 2007; Guneysel et al., 2009; Zhao et al., 2009). In addition, our previous data (Tavichakorntrakool et al., 2012) showed that *E. coli* isolates from kidney stones and urine were resistant to trimethoprim/sulfamethoxazole (SXT). SXT is a combined drug that consists of one part of trimethoprim to five parts of sulfamethoxazole. Its actions are anti-folate in the folate synthesis and metabolism (Brown, 2014). The previous study found that *Listeria monocytogenes* showed filamentation after exposure to trimethoprim (Minkowski et al., 2001). However, we studied in the *E. coli* isolates from the most compatible pattern of antimicrobial drug resistance. To better understand the SXT resistance mechanism and its related disorders, analyses of changes in the bacterial cell length is required. Therefore, the present study aimed to evaluate the effect of SXT disc distance on cell length of SXT-resistant *E. coli* (SREC).

Methodology

Ethics statement

This study was reviewed and approved by the Institutional Ethical Committee at Khon Kaen University.

Sample isolation and identification of bacteria

Seven SREC samples were isolated from stone and urine. All *E. coli* isolates were tested for antimicrobial susceptibility by the disc diffusion assay on Mueller Hinton agar (MHA). Five antimicrobial agents were tested including amikacin (30 µg), gentamicin (10 µg), cefotaxime (30 µg), ceftazidime (30 µg) and trimethoprim/sulfamethoxazole (SXT, 1.25/23.75 µg) (Oxoid Ltd., Basingstoke, England). Extended spectrum beta-lactamase (ESBL) -producing bacteria was determined by double-disc diffusion test according to the standard method of Clinical and Laboratory Standards Institute (CLSI, 2006). Seven SREC (susceptible to all antimicrobial agents except SXT) and non ESBL producer were used in this study.

Bacterial cell length evaluation

All *E. coli* isolates were cultured on MacConkey agar at 37°C for 24 hrs. The isolated colony was suspended and adjusted to 0.5 McFarland with normal saline. The bacterial suspension was streaked on MHA. A SXT disc was placed at the center of the plate and incubated at 37°C for 24 hrs. All 7 SREC isolates at the distances of 0, 5, 10 and 15 mm from the rim of SXT disc were picked up and suspended in 1 µL of normal saline on glass slide area (1.2 cm x 1.2 cm). After staining with 0.25% safranin O, the length of each cell in one field of view was measured under a light microscope (Nikon ECLIPSE 80i Microscope, Nikon Corporation, Japan).

Data analysis

All the quantitative data were reported as mean±standard error of mean (SEM). Differences among four sample groups were analyzed by using ANOVA with SPSS software (version 17.0) (SPSS Corporation; Chicago, IL). To test for correlations between two parameters, linear regression analysis was performed. P value < 0.05 was statistically significant.

Results

For quantitative change, we observed that the highest SXT concentration at the disc rim (0 mm) resulted in bacterial elongation, which was inversed at 15 mm SXT disc distance from the disc rim (Figure 1).

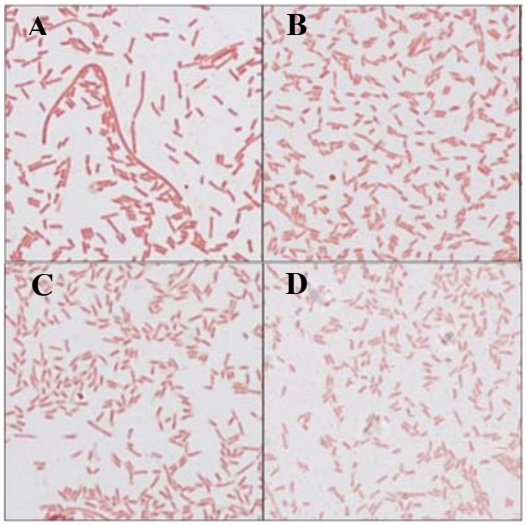

Figure 1 Effect of the serial distant changes from the rim of SXT disc at 0 (A), 5 (B), 10 (C) and 15 (D) mm on bacterial cell length.

The bacterial cell length of all SREC isolates was measured at the distances of 0, 5, 10 and 15 mm from the rim of SXT disc on MHA and expressed as mean±SEM.

Table 1 The cell lengths (µm) of all 7 SREC isolates at the distances of 0, 5, 10 and 15 mm from the rim of SXT disc.

Distance from the rim of SXT disc (mm)	Bacterial cell length (µm)	*p*-value
0	1.50±0.19	ND
5	1.10± 0.12	0.016
10	0.94± 0.05	0.002
15	0.82± 0.05	0.000

ND = non-detectable

When compared with SXT disc distance from the disc rim at 0 mm, the other distances showed statistically significant of *E. coli* cell length. Linear regression analysis showed significant negative correlation of bacterial cell length and the distance from the rim of SXT disc [r= –0.958, $p<0.05$] (Figure 2).

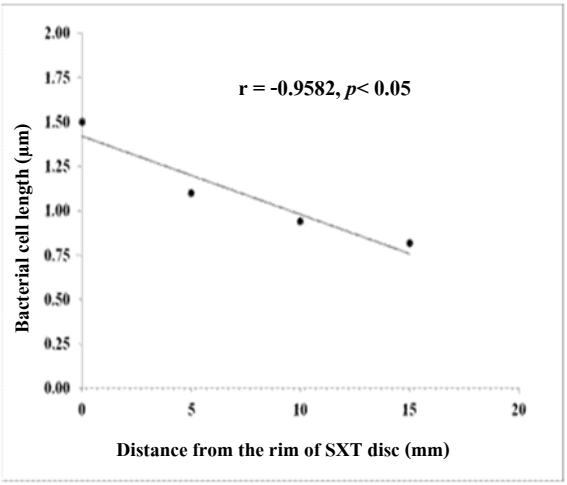

Figure 2 Correlation between bacterial cell length and distance from the rim of SXT disc (mm) on MHA.

Discussion and conclusion

We observed the negative correlation between bacterial cell length and the distance from the rim of SXT disc in the SREC isolates. The results from this study indicated that the SXT concentration had an effect on cell separation of the SREC isolates. This study was consistent with the previous reports, which demonstrated that cell elongation was found in *Listeria monocytogenes* (Minkowski et al., 2001) and *Enterobacter aerogenes* (Ingham et al., 2006) after exposure to trimethoprim. In addition, SXT inhibits dihydrofolatesynthase and dihydrofolatereductase, acts as the antifolate (Huovinen, 2001). While folate is needed by dividing cells to make thymine in nucleic acid synthesis. So, these bacterial cells lost of

the ability to form septa in cell division after expose to SXT.

In conclusion, the present study reveals that SXT had the effect on cell elongation of SREC isolates. It may imply that the SREC isolates can adapt themselves and survive in the stress condition.

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