COMPARISON BETWEEN EXPOSURE OF CIPROFLOXACIN AND CEFOTAXIME ON DEVELOPING OF *Escherichia coli* ESBL

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ABSTRACT

This study aimed to compare ciprofloxacin and cefotaxime exposure to develop ESBL producing Escherichia coli (E. coli). A total of 16 isolates of cefotaxime sensitive E. coli and ciprofloxacin were exposed to ciprofloxacin and cefotaxime for 14 days using the Kirby-Bauer antibiotic disc diffusion method. Colonies that grew on the edge of the inhibiting zone were exposed each day by the same method. Furthermore, we observed the occurrence of resistance to cefotaxime as ESBL screening test. Isolates were resistant, the following day the ESBL was confirmed by the Modified Double Disk Sinergy Test (MDDST) method using Cefotaxime (CTX), Ceftazidime (CAZ), Aztreonam (ATM), and Amoxilin Clavulanate (AMC) antibiotic discs. From 16 isolates of ESBL producing E. coli exposed to ciprofloxacin, it was obtained 4 (25%) to ESBL E. coli. ESBL production occurred after E. coli was exposed to ciprofloxacin on days 5, 6, 7, and 12. While those exposed to cefotaxime none becomes ESBL E. coli. There was no difference between ciprofloxacin and cefotaxime exposure to develop ESBL producing E. coli (p=0.101; Chi-square).

***Keywords**: E. coli ESBL; exposure of ciprofloxacin; exposure of cefotaxime; MDDST*

ABSTRAK

Penelitian ini bertujuan untuk membandingkan paparan siprofloksasin dan sefotaksim untuk mengembangkan Escherichia coli (E. coli) yang memproduksi ESBL. Sebanyak 16 isolat E. coli dan ciprofloxacin yang sensitif terhadap cefotaxime terkena ciprofloxacin dan cefotaxime selama 14 hari menggunakan metode difusi cakram antibiotik Kirby-Bauer. Koloni yang tumbuh di tepi zona penghambat terpapar setiap hari dengan metode yang sama. Lebih lanjut diamati terjadinya resistensi terhadap sefotaksim sebagai tes skrining ESBL. Isolat resisten, pada hari berikutnya ESBL dikonfirmasi oleh metode Modified Double Disk Sinergy Test (MDDST) menggunakan Cefotaxime (CTX), Ceftazidime (CAZ), Aztreonam (ATM), dan cakram antibiotik Amoxilin Clavulanate (AMC). Dari 16 isolat ESBL yang memproduksi E. coli yang terpapar ciprofloxacin diperoleh 4 (25%) dari ESBL E. coli. Menghasilkan ESBL terjadi setelah E. coli terpapar ciprofloxacin pada hari ke 5, 6, 7, dan 12. Sementara terkena cefotaxime, tidak ada yang menjadi ESBL E. coli. Tidak ada perbedaan antara paparan siprofloksasin dan sefotaksim untuk mengembangkan E. coli penghasil ESBL (p=0,101; Chi-square).

***Kata kunci**: E. coli ESBL; paparan siprofloksasin; paparan sefotaksim; MDDST*

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INTRODUCTION

Infections caused by ESBL-producing bacteria are considerable (Sturenburg & Mack 2003). Antibiotic-resistant bacteria are increasing in hospitals, especially in the Intensive Care Unit (ICU) (Paramythiotou & Routsi 2016). The use of beta-lactam antibiotics is considered as one of the risk factors of increasing incidence of resistant bacteria strains. This is because the beta-lactam group is considered the safest, and effective for the treatment of infectious diseases caused by bacteria (Bush 2009). Beta-lactam compounds inhibit bacterial cell wall synthesis by binding Penicillin Binding Protein (PBP), a peptidoglycan transpeptidase enzyme (Gallo & Puglia 2014) which catalyzes the last stage of bacterial cell wall formation (Bush 2009).

The number of infections caused by antibiotic resistant bacteria makes treatment more difficult (Dhillon & Clark 2011), since bacteria are usually resistant to many

other classes of antibiotics (Talan et al 2016). It is known that there are some things that become risk factors for infection by ESBL-producing bacteria. Second-generation cephalosporins (cefuroxime) therapy was reported to be a risk factor for infection by ESBL-producing bacteria with Odd Ratio (OR) according to Colodner by 10.1 (Colodner et al 2004) and by Talan of 21.42 (Talan et al 2016). Provision of prior third-generation cephalosporins therapy was a risk factor for infection by ESBL-producing bacteria by 15.8 (Colodner et al 2004). Paterson and Bonomo reviewed that previous cefotaxime administration did not make *E. coli* produce ESBL as opposed to FQ, aminoglycoside, cotrimoxazole and metronidazole (Paterson & Bonomo 2015).

Use of antibiotics can make normal flora resistant to the class of antibiotics. For example, giving beta-lactam antibiotics makes cell wall metabolism disturbed, so that muropeptides, as the result of the damaged cell wall fragments will increase. Under normal circumstances, the muropetide is recycled into the cell wall again. However, when there is interference metabolism of cell wall and muropeptides increase then through AmpC pathway bacteria increase expression betalactamase. The betalactamse enzyme hydrolyzes the beta-lactam ring so that bacterial resistance occurs to this class (Babic & Bonomo 2009, Zeng & Lin 2013).

The scientists found the phenomenon of cross resistance among different antibiotic classes (Talan et al 2016). This explains why fluoroquinolon class will be able to cause resistance to beta-lactam class. Administration of aminoglycoside class antibiotics, fluoroquinolone, beta-lactam, cotrimoxazole and tetracycline, are mentioned to be a risk factor. Paterson and Bonomo have reviewed several journals and concluded that the significant risk factors for infection by ESBL-producing bacteria are fluoroquinolone, aminoglycoside, cotrimoxazole and metronidazole (Paterson & Bonomo 2015).

This cross resistance may occur because the antibiotic resistance-encoding gene lies in the same plasmid (Moodley & Guardabassi 2009, Dolejska et al 2013), so that when plasmid conjugation occur in response to and adapt to certain antibiotic classes, the antibiotic resistance gene others carried along. The existence of a plasmid conjugation process occurring among bacteria receiving antibiotic exposure can lead to the spread of resistance genes. One plasmid can contain several different genes encoding resistance antibiotic classes. Among these genes are Plasmid Mediated Quinolone Resistance (PMQR), ESBL encoding genes, aminoglycoside resistance coding genes, and tetra-cyclines. Cross-resistance may occur between classes of antibiotics (Dolejska et al 2013, Jacoby et al 2014).

MATERIALS AND METHODS

Sixteen of non-ESBL *E. coli* isolates sensitive to cefotaxime and ciprofloxacin were exposed to ciprofloxacin (ciprofloxacin disc 5μg) and cefotaxime (cefotaxime disc 30μg) for 14 days using antibiotics disc diffusion method of Kirby-Bauer. Colonies growing on the edge of the inhibition zone were subcultured and reapplied daily with the same method. Furthermore, the observed occurrence of resistance to cefotaxime was considered as ESBL screening test (CLSI M100S 2016). It is said to be resistant if the inhibition zone diameter is ≤ 26 mm (CLSI M100S 2016). Furthermore, the cefotaxime-resistant isolates were tested for confirmation of ESBL using the Modified Double Disk Sinergy Test (MDDST) method using cefotaxime antibiotics (30 μg cefotaxime disc), ceftazidime (30 μg ceftazidime disc), aztreonam (30 μg aztreonam disc), and amoxilin/clavulanate (Amoxilin 20 μg/clavulanate 10 μg discs).

Amoxilin/clavulanate discs are placed in the middle for plates, other discs are placed around them, with a 25 mm center to center distance. Test was considered positive if there is expansion of disc zone cefotaxime, ceftazidime, and aztreonam discs toward amoxilin/ clavulanate disc (Kaur et al 2013).

RESULTS

From 16 isolates of non-ESBL *E. coli* exposed to ciprofloxacin developed 4 (25%) to ESBL *E. coli*. ESBL *E. coli* occurs after exposure to ciprofloxacin on days 4,5,7, and 12, whereas exposure to cefotaxime does not exist to ESBL *E. coli*.

Table 1. CIP and CTX sensitivity test results with Phoenix automatic dilution method and diffusion of Kirby-Bauer manual discs (n=18)

No	Sensitivity	Phoenix		Kirby-Bauer	
		CTX	CIP	CTX (%)	CIP (%)
1	Sensitive	18	18	18 (100)	16 (88.9)
2	Resistant	0	0	0	2 (11.1)

Table 2. *E. coli* resistance to CIP and CTX post recurrent CIP exposure by Kirby-Bauer method (n=16)

No	Anti-biotic	Exposure until days -							
		4		8		12		14	
		S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
1	CTX	16 (100)	0 (0)	12 (75)	4 (25)	12 (75)	4 (25)	12 (75)	4 (25)
2	CIP	16 (100)	0 (0)	16 (100)	0 (0)	16 (100)	0 (0)	16 (100)	0 (0)

Table 3. *E. coli* resistance to CIP and CTX post recurrent CTX by Kirby-Bauer method

No	Anti-biotic	Expssure until days -							
		4		8		12		14	
		S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
1	CTX	16 (100)	0 (0)	16 (100)	0 (0)	15 (93.8)	1 (6.2)	16 (100)	0 (0)
2	CIP	16 (100)	0 (0)	16 (100)	0 (0)	16 (100)	0 (0)	16 (100)	0 (0)

Table 4. ESBL confirmation test results from CTX resistant *E. coli* by MDDST

No	Antibiotic exposure	Exposure until days-							
		4		8		12		14	
		Non-ESBL (%)	ESBL (%)	Non-ESBL (%)	ESBL (%)	Non-ESBL (%)	ESBL (%)	Non-ESBL (%)	ESBL (%)
1	CTX	16 (100)	0 (0)	16 (100)	0 (0)	16 (100)	0 (0)	16 (100)	0 (0)
2	CIP	16 (100)	0 (0)	13 (81.2)	3 (18.8)	13 (81.2)	3 (18.8)	12 (75)	4 (25)

There were 2 (11.1%) isolates of ciprofloxacin resistant *E. coli* after confirmation by Kirby-Bauer disc diffusion method. Until the 8th day of exposure with ciprofloxacin, 4 (25%) isolates were found to be resistant to cefotaxime. Up to day 12 and 14 were found 4 (25%) isolates were cefotaxime resistant. Until the 14th day of ciprofloxacin exposure there were no isolates that became resistant to ciprofloxacin.

Until the 4th day and 8th, there were still no isolates resistant to cefotaxime. Resistance to cefotaxime was obtained on exposure to day 10, but the following day the isolates became sensitive again. So on the 14th day no isolates were found to be resistant to cefotaxime and ciprofloxacin.

Until the 4th day there is still no isolates that become ESBL. Until the 8th day, 3 (18.8%) of the isolates became ESBL. Three new ESBL isolates were obtained on days 6, 7, and 8. Until day 12 ESBL isolates did not increase. There were 4 (25%) ESBL isolates until day 14.

DISCUSSION

Actually, there were 5 isolates of *E. coli* exposed to CIP (1357PS, 1564US, 1590SS, 2015SS, and 2707PS) resistant to cefotaxime (Tabel 1.2) but become only 4 isolates (1357PS, 1564US, 2015SS, and 2707PS) confirmed as ESBL *E. coli*. While isolate 1590SS was not confirmed as ESBL *E. coli* as in the next day became sensitive to cefotaxime. Whether this phenomenon belongs to temporarily resistant is should be evaluated more detail.

Similarly, *E. coli* isolates (2056US) from the cefotaxime-exposed group was not proven to be ESBL *E. coli*. This isolate becomes sensitive again to cefotaxime in the next day. Occurrence of ESBL *E. coli* from CIP group appeared after exposure in days 5, 6, 7, and 12.

None of the isolates of the two groups became resistant to ciprofloxacin. No difference was found between ciprofloxacin and cefotaxime exposure to ESBL on *Escherichia coli* (p=0.101; Chi-square).

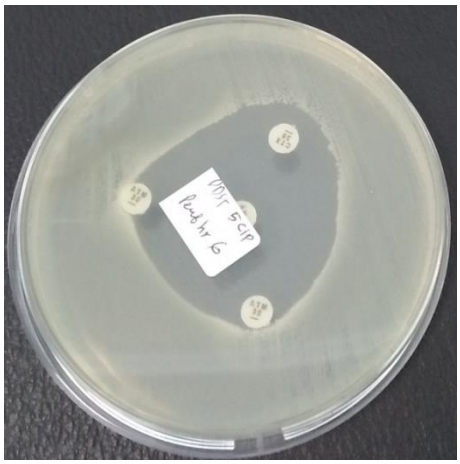

Fig. 1. Test day 6 MDDST isolate 1564US CIP group.

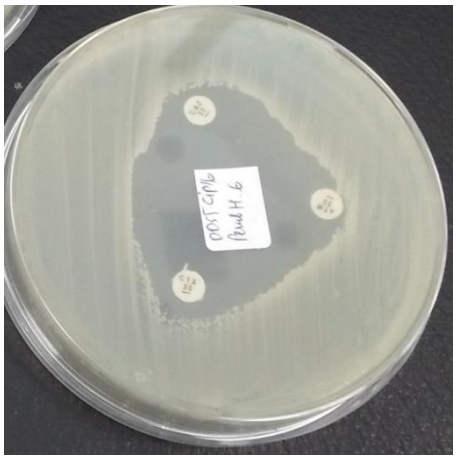

Fig. 2. Test day 7 MDDST isolates 2707PS group CIP.

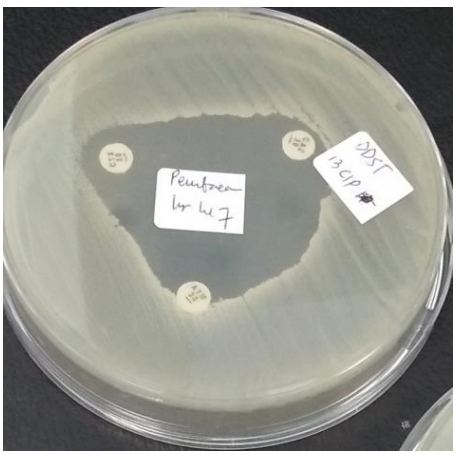

Fig. 3. Test day 8 MDDST isolate 2015SS CIP group.

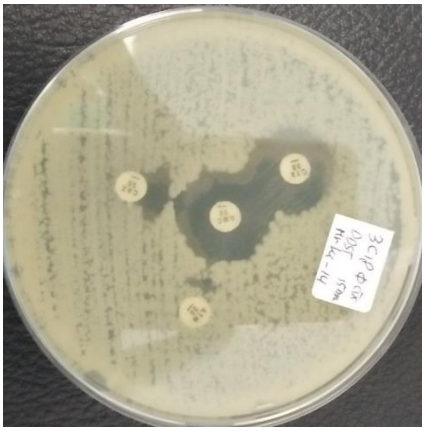

Fig. 4. Test day 14 MDDST isolate 1357PS group CIP.

The occurrence of *E. coli* ESBL after exposure to ciprofloxacin may be due to the transfer of plasmids from strains that have both plasmids containing the PMQR gene and ESBL-encoding genes. Isolates did not show early properties of resistancy to ciprofloxacin. This may be due to the fact that having only the PMQR gene does not make it resistant enough, since PMQR is not the main mechanism of resistance to ciprofloxacin (Moudgal & Kaatz 2009, Jacoby et al 2014). The presence of *E. coli* strains that have PMQR genes but their phenotypic properties are not resistant to fluoroquinolone is demonstrated by Fortini et al (2015).

CONCLUSION

There were 4 (25%) of 16 isolates of *E. coli* exposed to ciprofloxacin become *E. coli* producing ESBL. This occure was started from days 5, 6, 7, and 12. No *E. coli* is exposed to cefotaxime being *E. coli* producing ESBL. There was no difference in the occurence between ciprofloxacin and cefotaxime exposed group in developing ESBL *E. coli* (p=0.101; chi square). The use of ciprofloxacin in infectious diseases needs to be done with caution, especially after the fifth day of administration.

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