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 Amoxicillin 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

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
MATERIALS AND METHODS

Sixteen of non-ESBL E. coli isolates sensitive to cefotaxime and ciprofloxacin were exposed to ciprofloxacin (ciprofloxacin disc 5μg) and cefotaxime (ceftaxime 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 |  |  |  |
|----|-------------|--------|  |  |  |
|    |             |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 | Antibiotic | 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 | Antibiotic | Exposure 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 until days- |
|----|------------|----------------------|
|    |            | 4  | 8  | 12 | 14 |
|    |            | Non-ESBL (%) | ESBL (%) | Non-ESBL (%) | ESBL (%) | Non-ESBL (%) | ESBL (%) | Non-ESBL (%) | ESBL (%) |
| 1  | CTX        | 16 | 0 | 16 | 0 | 16 | 0 | 16 | 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 (Table 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).
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 resistance 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 occurred 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 occurrence 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.

REFERENCES

Babic M, Bonomo RA (2009). Mutation as a basis of antimicrobial resistance. Antimicrobial drug resistance mechanisms of drug resistance clinical and epidemiological aspects, 65-74
Bush K (2009). The importance of B lactamases to the development of new B lactams. Antimicrobial drug resistance: mechanisms of drug resistance, clinical and epidemiological aspect, p 135-44
CLSI M100S (2016). Performance standards for antimicrobial susceptibility testing. Twenty-fourth Informational Supplement, 118-21

Colodner R, Rock W, Chazan B, et al (2004). Risk factor for the development of extended-spectrum beta-lactamase-producing bacteria in nonhospitalized patients. Eur J. Clin. Microbiol Infect Dis., 163-7

Dhillon RHP, Clark J (2012). ESBLs: A clear and present danger? Critical Care Research and Practice. Available at https://www.hindawi.com/journals/ccrp/2012/625170/. Accessed November 11, 2016

Dolejska M, Villa L, Hasman H, Hansen L, Carottoli A (2013). Characterization of IncN plasmids carrying blaCTX-M-1 and qnr genes in Escherichia coli and Salmonella from animals, the environment, and humans. J. antimicrobe Chemother 68, 333-9

Fortini D, Fashae K, Villa L, Feudi C, Fernandez AG, Carattoli A (2015). A novel plasmid carrying blaCTX-M-15 identified in commensal Escherichia coli from healthy pregnant women in Ibadan, Nigeria. Journal of Global Antimicrobial Resistance 3, 9-12

Gallo G, Puglia AM (2014). Antibiotics and resistance: A fatal attraction. Antibiotics: Target, mechanisms, and resistance. Germany, Wiley VCH Weinheim, p 73-108

Jacoby GA, Strahilevitz J, Hooper DC (2014). Plasmid mediated quinolone resistance. NIH Public Access. Available at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4288778/. Accessed December 5, 2016

Kaur J, Chopra S, Sheevani, Mahajan G (2013). Modified double disc synergy test to detect ESBL production in urinary isolates of Escherichia coli and Klebsiella pneumoniae. Journal of Clinical and Diagnostic Research 7, 229-33

Moodley A, Guardabassi L (2009). Transmission of IncN plasmids carrying bla CTX-M-1 between commensal Escherichia coli in pigs and farm workers. Antimicrob Agents Chemother 53, 1709-11

Moudgal VV, Kaatz GW (2009). Fluoroquinlon resistance in bacteria. Antimicrobial Drug Resistance: mechanisms of Drug Resistance, Clinical and Epidemiological Aspect, 195-206

Paramythiotou E, Routsi R (2016). Association between infections caused by multi drug resistant gram-negative bacteria and mortality in critically ill patients. World J Crit Care Med 5, 111-20

Paterson DL, Bonomo RA (2015). Extended-spectrum bactamases: a clinical update. CLIN. MICROBIOL. REV. 18, 657-86

Talan DA, Takhar SS, Krishnadasan A, Abrahamian FM (2016). Fluoroquinolone-resistant and extended-spectrum ß-lactamase-producing Escherichia coli infections in patients with pyelonephritis. United States, Emerging

Sturenburg E, Mack D (2003), Extended-spectrum beta-lactamases: Implications for the clinical microbiology laboratory, therapy, and infection control. J. Infect. 47, 273-295

Zeng X, Lin J (2013). Beta-lactamase induction and cell wall metabolism in Gram-negative bacteria. Available at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC36-60660/. Accessed October 20, 2016