THE PATTERN OF ANTIBIOTIC PRESCRIPTION AND ANTIMICROBIAL RESISTANCE OF GUT FLORA *Escherichia coli* AT AISYIYAH HOSPITAL, BOJONEGORO

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ABSTRACT

Antimicrobial resistance (AMR) is the failure of antibiotic to kill bacteria and becomes ineffective in therapeutic purpose. The AMR bacteria is a major health problem worldwide and Indonesia is not exception. AMR is increased by two factors, higher antibiotic use and low compliance in infection control and prevention. WHO has recommended 7 bacterial indicators as point of view in surveillance, one of these bacteria is Escherichia coli. This study aimed to analyze the correlation between antibiotic use and resistance pattern of gut flora Escherichia coli. The study was conducted at Aisyiyah Hospital, Bojonegoro from June to October 2017. Total 101 patients from internal medicine and surgery department in this hospital were included in this study. Bacterial gut flora were tested against 12 antibiotics by disk diffusion test at the Department of Clinical Microbiology, Universitas Airlangga.. The results showed that the highest quantity of antibiotic use in internal medicine service was cefepime (40,50 DDD) and the highest resistance rate was ciprofloxacin, whereas in the surgical service it was ceftriaxone (132,75 DDD) with the highest E. coli resistance to amoxicillin-clavulanic acid. The antibiotics use has significant correlation against E. coli resistance on cefotaxime (p=0.046), ceftazidime (p=0.046), ceftriaxone (p=0.017), aztreonam (p=0.024), and cefepime (p=0.010).

***Keywords**: Antimicrobial resistance; Escherichia coli; antibiotics*

ABSTRAK

Resistensi antimikroba adalah kegagalan antibiotik untuk membunuh bakteri dan menjadi tidak efektif dalam tujuan terapeutik. Bakteri AMR adalah masalah kesehatan utama di seluruh dunia dan Indonesia tidak terkecuali. AMR meningkat oleh dua faktor, penggunaan antibiotik yang lebih tinggi dan kepatuhan yang rendah dalam pengendalian dan pencegahan infeksi. WHO telah merekomendasikan 7 indikator bakteri sebagai sudut pandang dalam pengawasan, salah satu dari bakteri ini adalah Escherichia coli. Penelitian ini bertujuan untuk menganalisis hubungan antara penggunaan antibiotik dan pola resistensi usus flora Escherichia coli. Penelitian ini dilakukan di Rumah Sakit Aisyiyah, Bojonegoro dari Juni hingga Oktober 2017. Total 101 pasien dari bagian penyakit dalam dan operasi di rumah sakit ini dilibatkan dalam penelitian ini. Flora usus bakteri diuji terhadap 12 antibiotik dengan uji difusi cakram di Departemen Mikrobiologi Klinik, Universitas Airlangga. Hasil penelitian menunjukkan bahwa jumlah tertinggi penggunaan antibiotik dalam layanan pengobatan internal adalah cefepime (40,50 DDD) dan tingkat resistensi tertinggi adalah ciprofloxacin, sedangkan dalam layanan bedah itu adalah ceftriaxone (132,75 DDD) dengan resistensi E. coli tertinggi terhadap asam amoksisilin-klavulanat. Penggunaan antibiotik memiliki korelasi yang signifikan terhadap resistensi E. coli pada sefotaksim (p=0,046), seftazidim (p=0,046), seftriakson (p=0,017), aztreonam (p=0,024), dan sefepim (p=0,010).

***Kata kunci:** Resistensi antimikroba; Escherichia coli; antibiotik*

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INTRODUCTION

The Europe of the health and economicburden states that deaths from resistant bacterial infections in Europe are estimated to be over 25,000 patients and according to a CDC report in the deaths in the United States is about 23,000 patients each year (WHO 2012, CDC 2013). The widespread of antibiotics use including their overuse and misuse induces the emergence of resistant bacteria through selective pressure and less compliance to infection control and prevention (Lestari et al 2008, Rice 2012, Taur & Show 2013).

E. coli was used as an indicator of antibiotic resistance surveillance as it is mostly found as bacterial gut flora in human beings (WHO 2014). These bacteria also indicate the appearance of resistance and potential widespread resistance to other gut flora will result in the emergence of a resistant population. It also can be easily and rapidly grown in vitro in the laboratory making it easy to be investigated (Todar 2008, Bartoloni et al 2006).

This study was conducted at Aisyiyah Hospital, Bojonegoro where no previous research has been done regarding the quantity data of antibiotics use and antibiogram. In relation to the rapid increase of Gram negative Enterobacteriaceae resistance, especially *E. coli* and the high trend of antibiotic usage at Asiyiyah Hospital Bojonegoro this research was focused to analyze the correlation between antibiotic prescription and resistance of gut flora *Escherichia coli*as as the first step to optimize the antibiotics usage.

MATERIALS AND METHODS

Study site and period. The study design was observational cross sectional on the resistance pattern of gut flora *E. coli*. Inclusion criterion was hospitalized patients for more than or equal to three days. A number of 101 adult patients from internal medicine and surgery departement participated in this study .The sample used was a rectal swab specimen taken while the patient was discharged from the hospital which was collected from June to October 2017.

Antibiotic prescription. Antibiotic prescription data were extracted from the medical records from each patients. Defined daily dose (DDD) per patient for each drug or drug category prescribed every quarter was calculated following the World Health Organization (WHO) anatomical therapeutic chemical (ATC) classification system of 2017

Antibiotical resistance *E. coli*. Sample test started with Gram staining, bacterial inoculation on Mac Conkey media to be incubated at 35-37°C for 24 hours. *E. coli* bacteria colonies showed growth with round and smooth reddish color. Furthermore, the samples identified as *E. coli* were tested for antibiotics sensitivity qualitatively with disk diffusion test.

Statistical analysis. All data were analyzed by using the statistic software packages SPSS version 18.0. Each antibiotic prescription and resistance pattern of *E. coli* was analyzed descriptively. Chi square test was used to analyze correlation between *E. coli* resistance pattern and quantity of antibiotic usage.The criterion for statistical significance was a p-value <0.05.

RESULTS

Characteristic data of research subjects are found in Table 1. Eighty samples were identified *E. coli* consisting of 41 (51.3%) patients in internal medicine departement and 39 (48.7%) of surgery department. Male patients were as many as 42 (52.5%) and female patients were as many as 38 (47.5%), with age mostly between 18-65 years, in as 56 patients (70.0%), with an average age of 54.47 years (range 17-82 years).

Bacterial identification data were found in Table 2. Based on identification through rectal swab specimens it was found that 80 samples were identified as *E. coli*, in which 77 (76.2%) was ESBL negative *E. coli* comprising 40 (51.9%) from internal medicine samples and 37 (48.1%) from sugery sample, while 3 samples were identified as ESBL positive *E. coli* that included 1 (33.3%) internal medicine samples and 2 (66.7%) surgery samples. Of the 21 samples identified as not *E. coli*, 19 samples were identified Gram negative non *E. coli* in which 17 samples were identified as Klebsiella sp, 1 sample of Pseudomonas sp. and 1 sample of Salmonella sp. Of the remaining 2 samples, there were no bacterial growth and both were surgical samples.

E. coli resistance data based on the type of health service is presented in Table 3. Eighty samples were identified as *E. coli* which then tested with antibiotic sensitivity of Kirby Bauer method with 12 types of antibiotic testing of amoxicillin-clavulanic acid (AMC), cefotaxime (CTX), ceftazidime (CAZ), ceftriaxone (CRO), aztreonam (ATM), cefepime (FEP), ciprofloxacin (CIP), amikacin (AK), meropenem (MEM), pipperacillin-tazobactam (TZP), fosfomycin (FOS), and cefoperazone-sulbactam (SCF). Twenty one samples could not be tested for antibiotic sensitivity because they were identified as not *E. coli*.

Resistance profiles of internal medicine samples showed that *E. coli* was most resistant to ciprofloxacin, and that from surgery sample to amoxicillin-clavulanic acid. Chi Square analysis showed that significant differences of *E. coli* resistance between samples from internal medicine and surgery to meropenem (p=0.001) and cephoperazon-sulbactam (p=0.035).

Table 1. Characteristics of research subjects

No	Characteristics of research subject	Description	Identified *E. coli* (N=80)	Identified Non *E. coli* (N=21)	Total Samples (N=101)	P-value
1	Type of Service	Internal medicine	41 (51.3%)	9 (42.9%)	50 (49.5%)	0.494
		surgery	39 (48.7%)	12 (57.1%)	51 (50.5%)	
2	Age	18 – 65 years	56 (70%)	16 (76.2%)	71 (70.3%)	0.576
		66 – 79 years	18 (22.5%)	5 (23.8%)	23 (22.8%)	
		80 – 99 years	6 (7.5%)	0 (0%)	6 (5.9%)	
3	Sex	Female	38 (47.5%)	9 (42.9%)	47 (46.5%)	0.074
		Male	42 (52.5%)	12 (57.1%)	54 (53.5%)	
4	Underwriting Status	BPJS	44 (55%)	8 (38.1%)	52 (51.5%)	0.195
		Other insurance	3 (3.8%)	0 (0%)	3 (3%)	
		Non Insurance	33 (41.2%)	13 (61.9%)	46 (45.5%)	

Table 2. Distribution of bacteria identification

No	Bacterial profile	Type of Service			P-value
		Internal Medicine	Surgery	Total	
1	No bacterial growth	0 (0%)	2 (3.9%)	2 (2%)	0.157
2	*Escherichia coli*				
	● *E. coli* (Non ESBL)	40 (80%)	37 (72.5%)	77 (76.2%)	0.494
	● *coli* (ESBL+)	1 (2%)	2 (3.9%)	3 (3%)	
3	Gram negative non *Escherichia coli*	9 (18%)	10 (19.7%)	19 (18.8%)	0.836
	Total	50 (100%)	51 (100%)	101 (100%)	

Table 3. Distribution of *E. coli* resistance data and chi square output between variables type of service with *E. coli* resistance pattern

No	Tested AB	Resistance		P-value
		Internal Medicine	Surgery	
1	AMC	18 (45%)	22 (55%)	0.468
2	CTX	15 (42.9%)	20 (57.1%)	0.279
3	CAZ	14 (48.3%)	15 (51.7%)	0.352
4	CRO	16 (48.5%)	17 (51.5%)	0.281
5	ATM	14 (45.2%)	17 (54.8%)	0.631
6	FEP	13 (43.3%)	17 (56.7%)	0.427
7	CIP	21 (52.5%)	19 (47.5%)	0.559
8	AK	3 (100%)	0 (0%)	0.193
9	MEM	4 (66.7%)	2 (33.3%)	0.001
10	TZP	3 (60%)	2 (40%)	0.068
11	FOS	5 (100%)	0 (0%)	0.050
12	SCF	0 (0%)	0 (0%)	0.035

Note: AB=Antibiotics; AMC=Amoxicilline-clavulanic acid; CTX=Cefotaxime; CAZ=Ceftazidime; CRO=Ceftriaxone; ATM=Aztreonam; FEP=Cefepime; CIP=Ciprofloxacin; AK=Amikacin; MEM=Meropenem; TZP=Piperacillin-Tazobactam; FOS=Fosfomycin; SCF=Cefoperazon-sulbactam

Antibiotic usage data based on the service is shown in Table 4. From 50 internal medicine patients involved in the study, 36 (72%) patients received antibiotics and 14 (28%) of patients did not receive antibiotics, while from 51 surgical patients, 49 (96%) received antibiotics and only 2 (4%) did not received. From the results of chi square analysis significant correlation was found between the type of health service and antibiotics usage.

Table. 4 Profile of antibiotic use in each type of health service

Type of Service	Antibiotics use		Total	P-value
	Yes	No		
Internal Medicine (N=50)	36 (72%)	14 (28%)	50	0.001
Surgery (N=51)	49 (96%)	2 (4%)	51	
Total	85	16	101	

Table 5. Distribution of antibiotic use in total DDD

No	Antibiotics	Internal Medicine		Surgery		Total	
		Frequency	DDD	Frequency	DDD	Frequency	DDD
1	CRO	15 (24.59%)	37.00	31 (44.29%)	132.75	46 (35.12%)	169.75
2	FEP	10 (16.39%)	40.50	7 (10.00%)	45.50	17 L12.98%)	86.00
3	ZOX	2 (3.27%)	5.00	9 (12.86%)	11.75	11 (8.40%)	16.75
4	FUR	8 (13.11%)	13.35	2 (2.86%)	3.34	10 (7.63%)	16.69
5	MTZ	1 (1.64%)	2.00	10 (14.27%)	43.34	11 (8.40%)	45.34
6	MEM	4 (6.56%)	18.00	2 (2.86%)	11.50	6 (4.58%)	29.50
7	CTX	0 (0.00%)	0	2 (2.86%)	4.75	2 (1.53%)	4.75
8	FOS	1 (1.64%)	2.00	0 (0.00%)	0	1 (0.76%)	2.00
9	CIP	6 (9.84%)	17.20	1 (1.44%)	4.50	7 5.34%)	21.70
10	AMX	2 (3.28%)	9.00	0 (0.00%)	0	2 (1.53%)	9.00
11	EMB	2 (3.28%)	5.83	0 (0.00%)	0	2 (1.53%)	5.83
12	AK	1 (1.64%)	3.00	1 (1.44%)	3.00	2 (1.53%)	6.00
13	LEV	4 (6.56%)	20.00	3 (4.26%)	8.00	7 (5.34%)	28.00
14	AMC	1 (1.64%)	4.50	0 (0.00%)	0	1 (0.76%)	4.50
15	PA	1 (1.64%)	1.00	0 (0.00%)	0	1 (0.76%)	1.00
16	GEN	0 (0.00%)	0	2 (2.86%)	9.55	2 (1.53%)	9.55
17	RIF	1 (1.64%)	1.50	0 (0.00%)	0	1 (0.76%)	1.50
18	INH	1 (1.64%)	1.00	0 (0.00%)	0	1 (0.76%)	1.00
19	DOX	1 (1.64%)	1.00	0 (0.00%)	0	1 (0.76%)	1.00
	Total	61 (100%)	181.88	70 (100%)	277.98	131 (100%)	459.86

Note: AMC=Amoxiciline-clavulanic acid; CTX=Cefotaxime; CRO=Ceftriaxone; EP=Cefepime; CIP=Ciprofloxacin; AK=Amikacin; MEM=Meropenem; EMB=Ethambutol; FOS=Fosfomycin; ZOX=Ceftizoxim, FUR=Cefuroxime, MTZ=Metronidazole; AMX=Amoxicillin; LEV=Levofloxacin; PA=Pipemidic Acid; GEN=Gentamicin; RIF=Rifampicin; INH=Isoniazid; DOX=Doxycycline

Antibiotic use data in total DDD is shown in Table 5. From the DDD calculation from 19 antibiotics used in 101 samples, we found that antibiotics with the highest use in internal medicine service was cefepime, which was in 10 samples with a total 40.50 DDD. Antibiotics with the highest use in surgery service was ceftriaxone, in 31 samples with a total 132.75 DDD. Results of Chi-square test showing correlation between sex and age with resistance *E. coli* are presented in Table 6. Sex correlated to *E. coli* resistance against piperacilin-tazobactam, while age correlated to E coli resistance to ciprofloxacin.

Results of partial effect analysis between antibiotic use (in DDD) and the type of service in the resistance pattern is presented in Table 9. The type of service relates to *E. coli* resistance to piperacillin-tazobactam, fosfomycin and cefoperazon-sulbactam, while total antibiotics usage in DDD that are associated with *E. coli* resistance to cefepime, amikacin, meropenem, fosfomycin, and cefoperazone-sulbactam.

DISCUSSION

This was a prospective analytic observational study aimed to analyze correlation between antibiotic use and gut flora *E. coli* resistance. Many studies involved pathogenic bacteria from clinical isolates and in some cases are limited to facultative bacteria.

Table 6. Correlation between sex and age with *E. coli* resistance

No	Antibiotics	P	
		Sex	Age
1	AMC	0.163	0.328
2	CTX	0.490	0.692
3	CAZ	0.137	0.416
4	CRO	0.244	0.485
5	ATM	0.171	0.586
6	FEP	0.362	0.387
7	CIP	0.397	0.047
8	AK	0.710	0.075
9	MEM	0.218	0.522
10	TZP	0.042	0.432
11	FOS	0.544	0.945
12	SCF	0.282	0.940

Note: AB=Antibiotics; AMC=Amoxicilline-clavulanic acid; CTX=Cefotaxime; CAZ=Ceftazidime; CRO=ceftriaxone; ATM=Aztreonam; FEP=Cefepime; CIP=Ciprofloxacin; AK=Amikacin; MEM=Meropenem; TZP=Piperacillin-Tazobactam; FOS=Fosfomycin; SCF=Cefoperazon-sulbactam

Table 7. Correlation of antibiotic use (in%) to *E. coli* resistance

No	Antibiotics Test	Sig.	R
1	AMC	0.351	0.106
2	CTX	0.014	0.273
3	CAZ	0.046	0.224
4	CRO	0.017	0.266
5	ATM	0.024	0.252
6	FEP	0.010	0.285
7	CIP	0.269	0.125
8	AK	0.322	0.112
9	MEM	0.830	0.024
10	TZP	0.937	0.009
11	FOS	0.965	0.005
12	SCF	0.643	0.053

Note: AB=Antibiotics; AMC=Amoxicilline-clavulanic acid; CTX=Cefotaxime; CAZ=Ceftazidime; CRO=Ceftriaxone; ATM=Aztreonam; FEP=Cefepime; CIP=Ciprofloxacin; AK=Amikacin; MEM=Meropenem; TZP=Piperacillin-Tazobactam; FOS=Fosfomycin; SCF=Cefoperazon-sulbactam

Table 8 Correlation between total DDD on *E. coli* resistance

No	Tested AB	Sig.	R
1	AMC	0.059	0.212
2	CTX	0.003	0.327
3	CAZ	0.010	0.285
4	CRO	0.004	0.322
5	ATM	0.007	0.297
6	FEP	0.000	0.389
7	CIP	0.014	0.274
8	AK	0.314	0.114
9	MEM	0.413	0.093
10	TZP	0.462	0.083
11	FOS	0.528	0.072
12	SCF	0.248	0.131

Note: AB=Antibiotics; AMC=Amoxiline-clavulanic acid; CTX=Cefotaxime; CAZ=Ceftazidime; CRO=Ceftriaxone; ATM=Aztreonam; FEP=Cefepime; CIP=Ciprofloxacin; AK=Amikacin; MEM=Meropenem; TZP=Piperacillin-Tazobactam; FOS=Fosfomycn; SCF=Cefoperazon-sulbaktam, DDD=Defined Daily Dose

Table 9. Partial effect of antibiotics usage (in DDD) on the type service on *E. coli* resistance

No	Tested AB	OR (Odds Ratio)		B (regression coefficient)	
		Type of service	Total DDD	Type of service	Total DDD
1	AMC	0.641	0.843	-0.445	-0.171
2	CTX	0.287	0.575	-1.250	-0.554
3	CAZ	0.479	0.775	-0.737	-0.255
4	CRO	0.461	0.831	-0.774	-0.186
5	ATM	0.485	0.870	-0.724	-0.139
6	FEP	0.562	1.071	-0.576	0.068
7	CIP	0.351	0.968	-1.048	-0.033
8	AK	0.936	1.462	-0.067	0.380
9	MEM	0.167	1.096	-1.792	0.091
10	TZP	2.372	0.747	0.864	-0.292
11	FOS	1.406	2.961	0.340	1.085
12	SCF	3.373	1.056	1.216	0.055

Note: AB=Antibiotics; AMC=Amoxicilline-clavulanic acid; CTX=Cefotaxime; CAZ=Ceftazidime; CRO=Ceftriaxone; ATM=Aztreonam; FEP=Cefepime; CIP=Ciprofloxacin; AK=Amikacin; MEM=Meropenem; TZP=Piperacillin-Tazobactam; FOS=Fosfomycn; SCF=Cefoperazon-sulbactam, DDD=Defined Daily Dose

Antibiotics use does not only affect the pathogenic bacteria, but also the human normal microflora including *E. coli*. It is important to carry out further investigation because these bacteria can colonize and potentially transfer resistance to human pathogenic bacteria (Barbosa & Levy 2010). From June to October 2017, there were 101 patients who met the inclusion criteria. From Table 1 on patient's characteristics, there were no significant differences between internal medicine and surgical services (p=0.494), sex (p=0.074), age (p=0.576) and insurance status (p=0.195). This reflects that the randomization technique was quite in this study.

From 101 rectal swab specimens, 80 samples were identified as *E. coli* in which *E. coli* producing ESBL was in 3 samples and the remaining were on ESBL *E. coli*. The analysis was performed only in patients with *E. coli* isolates, aiming to reduce the bias of analysis due to differences in bacterial species.

Analysis of correlation between age and resitance pattern of E.coli by chi square test revealed that age had a significant correlation with *E. coli* resistance to ciprofloxacin (Table 6). This is possible because of age restriction related to of fluoroquinolone prescription including ciprofloxacin, thus minimizing the exposure of ciprofloxacin in certain age groups. A study by Arce et al (2011) found that age, sex and source of infection were the determinants of *E. coli* resistance modulation. Both males and females show enhanced resistance as age increases. Resistance in females tends to be constant or elevates slightly during delivery and increases rapidly in the premenopausal period. Males show increased resistance at pubertal age. Although the effect of age on increased resistance can only be explained in terms of the higher exposure to antibiotics (Arce et al 2011). Livemore et al (2003) suggested that the prevalence of ciprofloxacin-resistant *E. coli* was closely related to male sex with bacteremia, slightly correlated with age, and the peak resistance was in 15-44 age group.

The result of descriptive analysis on*E. coli* resistance pattern on 12 tested antibiotics revealed that the highest resistance in internal medicine department was to ciprofloxacin while that in surgery department was to amoxicillin-clavulanic acid (Table 3). Previous study (Arce et al 2011) also showed similar results. Amoxicillin-clavulanic acid and ciprofloxacin-resistant E coli were the first of 100,000 *E. coli* isolates tested. This is related to the overuse of both antibiotics in the community, which may be due to the presence of parenteral forms, amoxicillin-clavulanic acid and ciprofloxacin also available orally, so that their use is not limited only to hospital setting, but also to communities (Pedrera V et al 2004, Arce et al 2011). In addition, broad-spectrum antibiotic use is widely reported as a major cause of increasing resistance (Michael et al 2014) and it is well known that both amoxicillin-clavulanic acid and ciprofloxacin have broad spectrum activity.

From the sensitivity profile of 41 samples from internal medicine departement, *E. coli* was mostly sensitive to piperacillin-tazobactam, amikacin, and cefoperazon-sulbactam, while from 39 samples from surgery department *E. coli* was most sensitive to fosfomycin. A study by Arce et al (2011) also showed similar results. This is possible because amikacin and fosfomycin are potent antibiotics with narrow spectrum activity, used only in hospital setting rather than first-line therapy, so that selective pressure effect is small due to limited use (Arce et al 2011). High sensitivity of piperacillin-tazobactam and cefoperazon-sulbactam was possible because of their limited use, even not used in patients involved in this study (Table 5).

In this study, the ATC antibiotic code used in all samples of 9 groups with 19 different class of antibiotics. Based on the calculation of total DDD from 101 patients found that the highest of antibiotics use in internal medicine services was cefepime of 40.50 DDD, whereas in surgical service the highest was ceftriaxone of 132.5 DDD (Table 5). The widely prescription of ceftriaxone may be due to a profile of tissue penetration, a broad spectrum activity and low toxicity, so it can be used as a therapy for treating life-threatening infections, especially if the causative organism has not been isolated previously (Pottinger et al 2014). Cefepime is a fourth generation cephalosprorin with clinical benefits similar to that of the third generation but with better activity against P. aeruginosa (Pottinger et al 2014). In addition to the consideration of pharmacological activity, the high use of third and fourth generation cephalosprorins is also possible in health insurance status. Patients with BPJS status tend to be higher in number than patients with private or non-insurance status (Table 1). Ceftriaxone and cefepime are listed in a national formulary with no specific restriction on their use.

Descriptive analysis of antibiotic usage in both types of health services showed that the use of antibiotics in surgical services was higher than in internal medicine, ie, 96% vs 72% (Table 4). This is because the administration of antibiotics in surgical services is not only intended as causative therapy of infectious diseases but also as prophylaxis.

Several previous studies have been conducted to analyze the effect of quantity of antibiotic prescription on *E. coli* resistance. In the Netherlands, elevated *E. coli*-resistant norfloxacin correlated with an increase in fluoroqunolone prescribing in urinary tract infections (Goettsch et al 2000). A 2006-2008 surveillance study involving 4 general hospitals in Singapore showed similar results, cross-correlation analysis demonstrated possible associations between prescription of fluoroquinolones and ciprofloxacin-resistant *E. coli* (R2=0.46), fluoroquinolones and ceftriaxone-resistant *E. coli* (R2=0.47), and carbapenems and imipenem-resistant Acinetobacter spp. (R2=0.48) (Li-Yang et al 2010)

Regression test was used to analyze the effect of antibiotic usage quantitatively against *E. coli* resistance. The results was found that the quantity of antibiotic use in DDD had a positive effect on *E. coli* resistance on cefotaxime (p=0.003), ceftazidime (p=0.010), ceftriaxone (p=0.004), aztreonam (p=0.007), cefepime (p=0.000), and ciprofloxacin (p=0.014) (Table 8). When viewed from the antibiotic use profile (Table 5), where the highest use of ceftriaxone was in the surgery department and cefepime in internal medicine departement, this reinforces the results of this regression analysis. It has been recognized that cefotaxime, ceftazidim, aztreonam and cefoperazone - sulbactam have the same core structure as ceftriaxone and cefepimeie the ß-lactam ring that allows cross resistance against the four antibiotics of the tested. DDD antibiotics also affect the resistance of *E. coli* to ciprofloxacin. This is possible because of transferable resistance, clonal spread and mutation due to selection pressure by antibiotics usage (Kuntaman et al 2005).

Direct correlation between the antibiotic usage quantitatively and increased resistance can not be easily determined, although widely use of antibiotics is the major cause of resistance. On the other hand, there are many factors that influence resistance, such as cross-selection, spread, antibiotic residues, resistant gene transfer, infection control, patient transfer in a health institution, community mobility and socioeconomic factors (Alekshun & Levy 2000, Barbosa & Levy 2010). In short, resistance phenomena can be focused on two factors, ie the use of antibiotics as agents that have selective activity and the ability of bacteria to propagate their resistant genes through various mechanisms either through plasmids, extracromosomal elements or bacteriophage (Levy 2002). However, this study did not determine the effect of other factors on *E. coli* resistance.

Quality analysis becomes important to determine prudence in the use of antibiotics. Prudent use of antibiotics is their use appropriate to the cause of infection with optimal dosing regimens, optimal duration of administration, minimal side effects and minimal impact of the emergence of resistant bacteria. Prudent use of antibiotics require policy restrictions in its application (Kemenkes RI 2015).

The results of quantity and quality analysis on effect of antibiotic use againts *E. coli* resistance encouraged the establishment and implementation of guidelines for antibiotics use at Aisyiyah Hospital Bojonegoro. The aim of prudent use antibiotic is to optimize therapeutic effect of the antimicrobial agent while minimizing the development of antimicrobial resistance. Its implementation required the role and cooperation of clinicians, clinical microbiologists, pharmacists and nurses.

CONCLUSION

Antibiotics use has been shown to have significant effect on *E. coli* resistance to cefotaxime, ceftazidime, ceftriaxone, aztreonam, and cefepime. The results of quantity and quality analysis on the effect of antibiotic use on *E. coli* resistance encourage the establishment and implementation of guidelines for antibiotics use at Aisyiyah Hospital Bojonegoro.

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