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Research Article

EVALUATION OF ANTIBACTERIAL ACTIVITY AND DETERMINATION AMOXICILLIN CONCENTRATION ON GENERIC AND BRANDED PRODUCTS

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ABSTRACT

Objective: The aims of this research were to evaluate the antibacterial activity and to determine the concentration of seventeen generic and branded amoxicillin products.

Methods: Concentrations of generic and branded amoxicillin were determined using high performance liquid chromatography (HPLC) method. Antibacterial activity of active compound were determined using diffusion method.

Results: The amoxicillin concentration of all sampels (generic and branded) eligible to Indonesian Pharmacope IV edition's requirement which was 90-120%. Both products, generic as well as branded products showed similar antibacterial activity against *Staphylococcus aureus* ATCC 6538, *Streptococcus pneumonie* ATCC 16491, and *Escherichia coli*.

Conclusion: The generic and branded amoxicillin had no significant concentration difference as well as antimicrobial activity.

Keywords: Evaluation, Determination, Antibacterial, Amoxicillin.

INTRODUCTION

Antibiotics are the most frequently prescribed drugs among hospitalized patients especially for infectious diseases[1-3]. The usefulness of antibiotics was showed at period 1998-2000, the number of daily doses of antibiotics per 1000 inhabitants was 30.7 with a cost of 47.18 euros/1000 inhabitants per day. The Public National Health Care System prescribe that in Spain during the year 2004, the number of 25.61 million containers of combinations of penicillins, macrolides, fluorquinolones, and other betalactams, with a total cost of 336.12 million euros. By pharmaceutical specialties or drug products in the Valencian Community (Spain), amoxicillin and the association amoxicillin-clavulanic acid accounted for 67.8% of all prescriptions and 59.4% of the global price[4].

In Indonesia, survey of antibiotic usage showed that the antibiotics were prescribed by private practice (36%), healthcare centers (29%), doctors in public hospitals (12%), by nurses and midwives (6%), and self-medicated by 17% of the individuals[5]. Study on the antibiotic usage of adult patient in healthcare centers on Yogyakarta (Indonesia) at January until April 2010 by Hadi showed that, amoxicillin prescription still being the first choice antibiotic which as 64.53%, and then followed by ampicillin 11.31%, co-trimoxazole 15.90%, chloramphenicol, 0.61%, metronidazole 2.75% and tetracycline 4.89%[6].

The problem due to antibiotic consumption is antibiotic resistance of the pathogen microorganisms. Some of microorganisms were resistant to amoxicillin, for example *S. aureus, E. coli*, and *S. pneumonie*. *S. aureus* strains, 53.3% were resistant to amoxicillin, 60.0% to penicillin G, 50% to ampicillin, and 13.3% to amoxicillin/clavulanic acid[7]. Study on antimicrobial drug resistance in *E. coli* from Humans and Animals food in United States showed that *E. coli* were resistant to amoxicillin/clavulanic acid (5.6%)[8]. In 1974, the incidence of penicillin resistance to *S. pneumonie* in Australia and New Guinea was reported to be 12%; by 1980, it had reached 33%[9,10].

Amoxicillin is one of the semi-synthetic penicillins discovered by Beecham scientists. Sales of the branded amoxicillin in the US were approximately US$34 million (€22.83 million) in 1999. The average branded prescription cost was over 3 times than the average generic cost in 2006 (US$111.02 vs. US$32.23)[11].

Generic products are 'copies' of patented drugs and can be marketed at low price following patent expiration of the branded products. The aim of generic drug development is to lower public health costs[11,12]. But still there is some doubt regarding to generic products usage compared to branded products[13]. Based on research investigated by Del Tacca (2009), three of four generic amoxicillins had 80-95% concentration profile to branded amoxicillin. This result linier to statement of FDA[11]. Statistic data from the USA has shown that branded manufacturers do not compete on cost once generic products as an competitors become available, because generic drugs are typically more cheap than their corresponding branded, competition from generic drugs can deliver large savings to consumers[14,15]. Therefore, the aim of this research is to evaluate antibacterial activity and determine amoxicillin concentration of generic and branded products, so the effectivity and quality of generic and branded products can be compared.

METHODS

Amoxicillin Products

Amoxicillin 500 mg, generic and branded products were obtained from pharmacies in West Java, Bandung, Indonesia.

Microorganisms

Bacteria used in this study were gram positive bacteria: *S. aureus* ATCC 6538 and *S. pneumonie* ATCC 16491. While Gram negative bacteria: *E. coli*. The pathogen bacteria were obtained from Sanbe Pharma Laboratorium, West Java, Indonesia.

Chemical Analysis

Chemical analysis included the qualitative analysis and quantitative analysis.

- **Qualitative analysis**

Oualitative analysis include the physical, weight uniformity, organoleptics, and identification analysis

- **Quantitative analysis**

Quantitave analysis using HPLC methods with stationary phase U-Bondopak C18 colom. Mobile phase used was KH_2PO_4 in 2 L aquadest at pH 5.0±0,1 with adding KOH 45%b/b solution. The amoxicillin concentration using following calculation:

$$\text{Amoxicillin concentration} = \frac{Au \times Ws \times Wc}{As \times Wu \times 500} Cs\%$$

Au = Area under curve amoxicillin of analyzed solution; As = Area under curve amoxicillin of standard solution; Ws = weight standard of amoxicillin (mg); Wu = weight analyzed sample (mg); Wc = average weight/capsul (mg); Cs = concentration of standard amoxicillin.

Study of generic and branded amoxicillin activities

Antibacterial activities of generic and branded amoxicillin was studied using agar difussion method.

Statistic analysis

The data statistically analyzed using ANOVA. The aim of this analysis is to confirm the result accuracy of the evaluation of concentration and antibacterial activity of amoxicillin 500 mg from the 17 pharmaceutical industries that produce antibiotics.

RESULTS AND DISCUSSION

Chemical analysis

Chemical analysis was used to evaluate amoxicillin concentration according on its lable. Amoxicillin concentration was analyzed on 7 generic products and 10 branded products using High Performance Liquid Chromatography (HPLC). Both generic products as well as branded products showed convenient concentration to Indonesian Pharmacope IV provision, which is 90-120% (Figure 1).

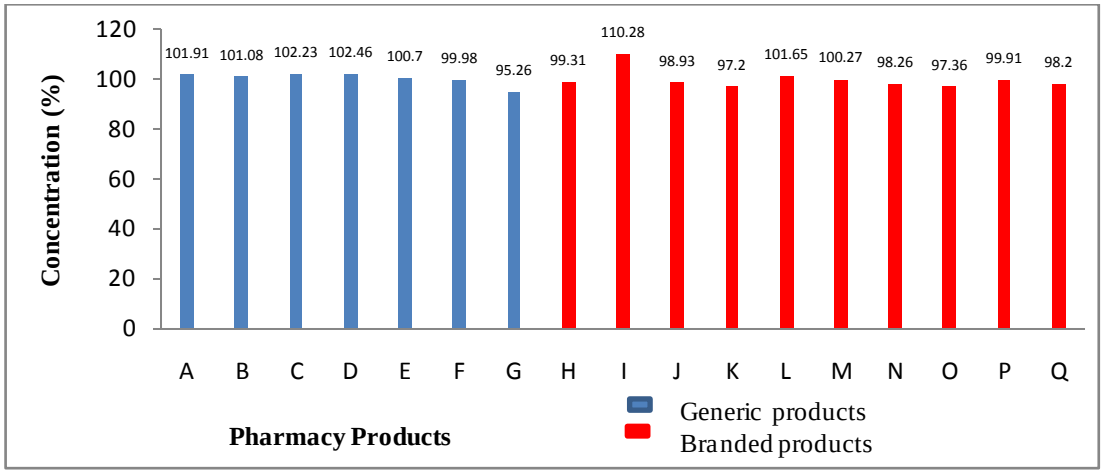
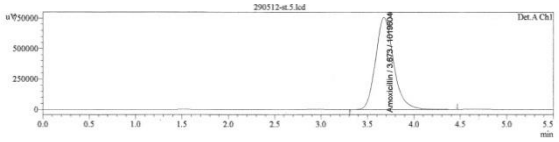
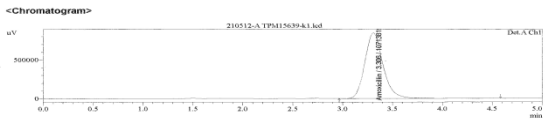
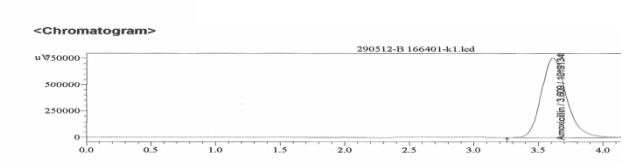
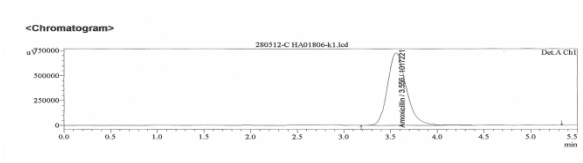
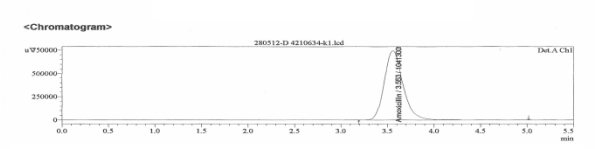
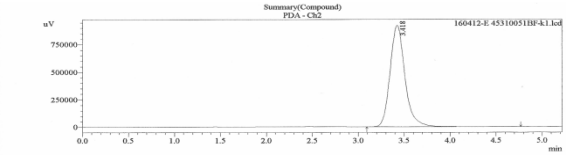
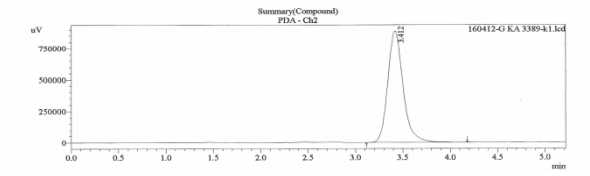


Fig. 1: Concentration of amoxicillin samples

Table 1 showed comparation of HPLC chromatogram profile between standard, generic and branded amoxicillin products.

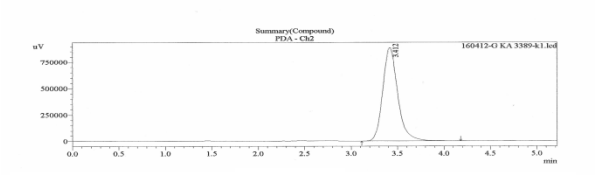
Table 1: HPLC chromatogram profile between standard, generic, and branded amoxicillin products

Chromatogram	
	
Standard Retention time : 3.680; AUC : 10199787; Concentration (%) : 100.31	Sample A Retention time : 3.340; AUC : 10714149; Concentration (%) : 105.50
	
Sample B Retention time : 3.608; AUC : 10273923; Concentration (%) : 101.06	Sample C Retention time : 3.556; AUC : 10126185; Concentration (%) : 102.11
	
Sample D Retention time : 3.553; AUC : 10382482; Concentration (%) : 102.33	Sample E Retention time : 3.416; AUC : 10380236; Concentration (%) : 100.73



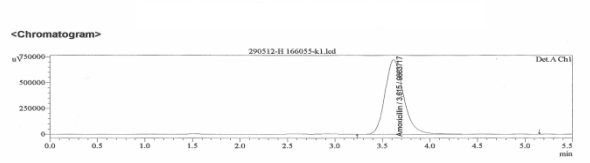
Sample F

Retention time : 3.410; AUC : 9922058; Concentration (%) : 95.281



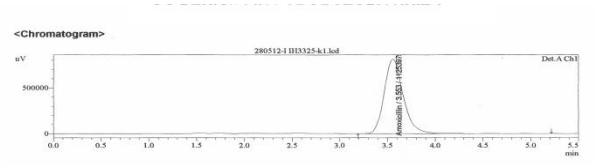
Sample G

Retention time : 3.410; AUC : 9922058; Concentration (%) : 95.281



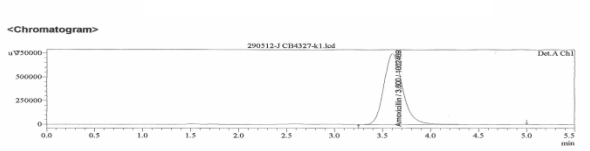
Sample H

Retention time : 3.613; AUC : 9865475; Concentration (%) : 97.77



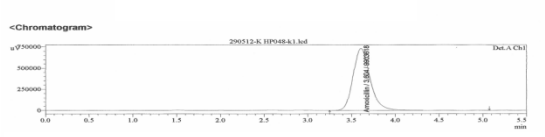
Sample I

Retention time : 3.551; AUC : 11246185; Concentration (%) : 110.85



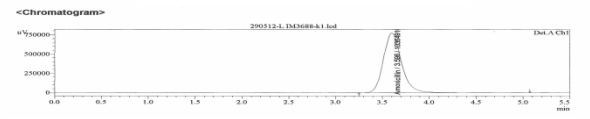
Sample J

Retention time : 3.599; AUC : 10015560; Concentration (%) : 98.91



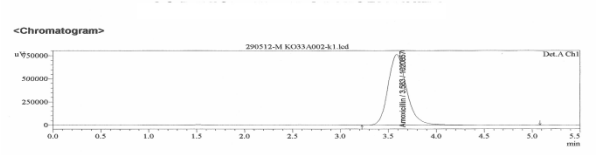
Sample K

Retention time : 3.603; AUC : 9898344; Concentration (%) : 97.17



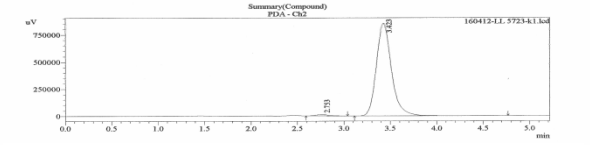
Sample L

Retention time : 3.593; AUC : 10327921; Concentration (%) : 101.63



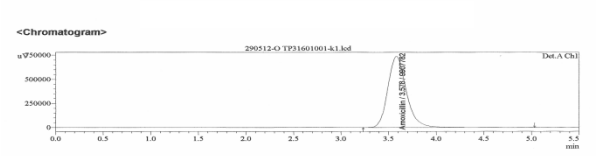
Sample M

Retention time : 3.580; AUC : 10167068; Concentration (%) : 100.24



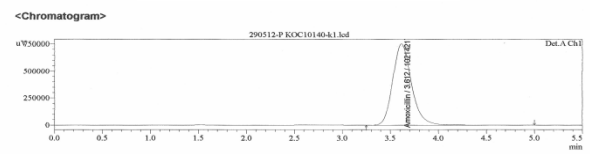
Sample N

Retention time : 3.422; AUC : 9776615; Concentration (%) : 94.217



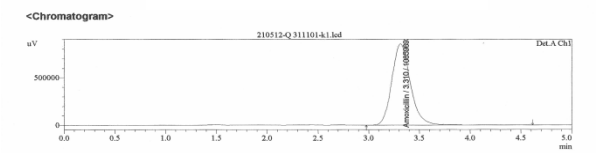
Sample O

Retention time : 3.580; AUC : 9897510; Concentration (%) : 97.34



Sample P

Retention time : 3.611; AUC : 10245575; Concentration (%) : 95.47



Sample Q

Retention time : 3.310; AUC : 10867245; Concentration (%) : 98.14

Antibacterial Activites of generic and branded products

The lowest antibacterial activity of generic amoxicillin against *S. aureus* ATCC 6538 was shown by G sample 99,37% and the highest activity was shown by D sample 101,28%. The lowest activity of branded products was shown by J sample 99,58% and the highest activity by P sample 100,95% (Figure 2). This result showed that generic products as well as branded products had no significant difference against *S. aureus* ATCC 6538.

Antibacterial activity of generic and branded amoxicillin showed had no significant difference against *E. coli*. The highest activity of generic amoxicillin was shown by E sample 101,11% and the lowest activity was shown by sample B 96,54%. The branded products showed the lowest activity on I sample 99,34% and the highest activity on H sample 100,64% (Figure 3).

Bioassay of amoxicillin against *S. pneumonie* ATCC 16491 was determined using two times concentrations of similar with concentration used to againsts *S. aureus* ATCC 6538 and *E. coli*. The clear area was expected to be obtain from this concentration and to confirm the accuracy of antibacterial activity of amoxicillin 1000 mg against *S. pneumonie* ATCC 16491.

The concentration of generic amoxicillin had the highest activity which shown by F sample, 100,67% and the lowest activity by B sample, 99,11%. The highest activity of concentration of branded amoxicillin was shown by J, O, and P sample, 100,22% and the lowest activity by H sample, 99,56% (Figure 4).

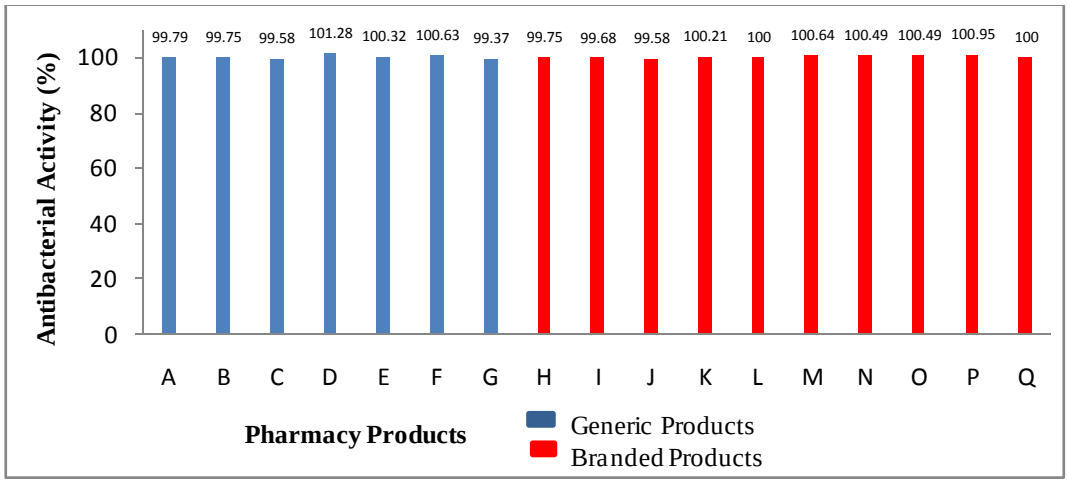


Fig. 2: Antibacterial activity of amoxicillin against *S. aureus* ATCC 6538

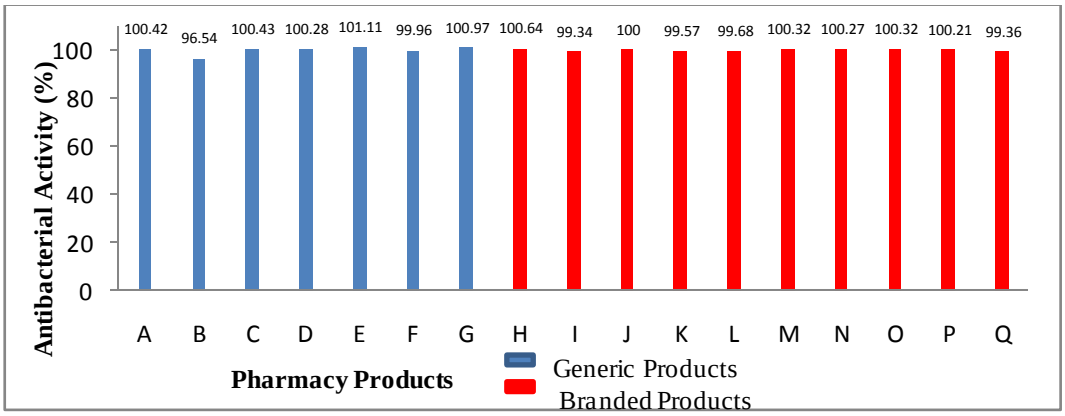


Fig. 3: Antibacterial activity of amoxicillin against *E. coli*

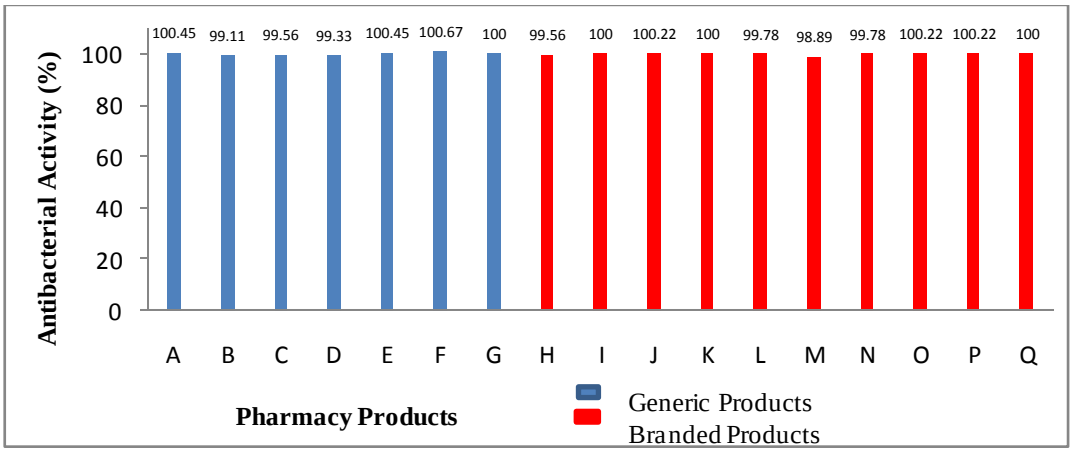


Fig. 4: Antibacterial activity of amoxicillin against *S. pneumonie* ATCC 16491

Amoxicillin still being drug of choice within the class because it has better pharmacokinetic than other β-lactam antibiotics for the treatment of infections due to susceptible organisms[16].

From the quantitative analysis and antibacterial activity study, both generic and branded amoxicillin active against *S. aureus* ATCC 6538, *E. coli,* and *S. pneumonie* ATCC 16491. These results showed there is unnecessary doubting the antibiotics effectivity, particularly generic and branded amoxicillin. Especially for generic products still become the drug of choice for the community with weak economic, hence can increasing the obedience of patient therapy and completeness of the therapy.

CONCLUSION

Generic and branded amoxicillin products showed similar amoxicillin concentration and fill requirement at Indonesian Pharmacope IV. The potency antibacterial activity of generic and branded amoxicillin products were 95-105% and had no significant different. Both products had no resistance against *S. aureus* ATCC 6538, *S. pneumonie* ATCC 16491, and *E. coli*.

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