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Antibacterial Effects of Nine Brands of Ciprofloxacin Tablets Available in Dar es Salaam

R.S. MALELE[1], K.D. MWAMBETE*[2] AND L.Q. HASSANALI[3]

[1]*Department of Pharmacognosy, School of Pharmacy, Muhimbili University of Health and Allied Sciences, P.O. Box 65085, Dar es Salaam, Tanzania.*
[2]*Department of Pharmaceutical Microbiology, School of Pharmacy, Muhimbili University of Health and Allied Sciences, P.O. Box 65013, Dar es Salaam, Tanzania.*
[3]*TMJ Hospital-Mikocheni, P.O. Box 20439, Dar es Salaam, Tanzania.*

Nine brands of ciprofloxacin tablets available in Dar es Salaam City were assayed for antibacterial effects against three strains of bacteria namely *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* using the Kirby-Bauer disc diffusion method. The ciprofloxacin brands were randomly coded with letters A to I for commercial protection. The sampled tablets were pulverized and dissolved in 0.1M HCl. At 0, 15, 30, 45 and 90 min, and 24 h, 20 µl aliquots were drawn and embedded onto 5 mm diameter Whatman filter papers. Equal volume of 0.1M HCl and commercially available ciprofloxacin disc (1 µg) were used as negative and positive controls, respectively. Results of the zones of inhibition at time 0 showed that none of the brands had comparable antibacterial effects as the positive control. The zones of inhibition produced by 3 brands (D, H and I) against *E. coli* were below specified limits (30-40 mm) at all assay times. The antibacterial effects of ciprofloxacin brands against *S. aureus* were only significant at 24 h. Brand A, which was the most expensive, exhibited the most potent antibacterial effect against test bacteria. Positive correlation between antibacterial effect and price was observed (R = 0.119; p = 0.290). High prevalence (44.4%) of poor quality ciprofloxacin tablets was observed. We recommend enforcement of post-market surveillance of medicines and more stern measures be imposed to prevent entry of poor quality drugs into Tanzania.

Key words: Antibacterial effect, inhibition zone, poor quality drugs

INTRODUCTION

Disease-causing microbes that have become resistant to drug therapy are an increasing public health problem [1]. Urinary tract infections (UTI), gonorrhoea, tuberculosis, and childhood ear infections are some of the bacterial diseases that have become difficult to treat with antimicrobial drugs due to widespread drug resistance. Microorganisms use diverse mechanisms to acquire drug resistance like horizontal gene transfer (plasmids, transposons and bacteriophages), recombination of foreign DNA in bacterial chromosome and mutations in different chromosomal loci [2, 3].

Ciprofloxacin is the most frequently prescribed fluoroquinolone for UTIs because of its availability in oral (tablet and suspension) and intravenous formulations [4]. It has shown an excellent activity against pathogens commonly encountered in complicated UTIs. It has good oral absorption and is rapidly excreted from the body under normal conditions [5, 6]. Resistance to fluoroquinolones, including ciprofloxacin, has increased markedly since their introduction for the treatment of UTIs [4-8]. The spectrum of poor quality drugs is particularly wide, ranging from a near precise copy of a genuine product to the extreme case of a drug product with none of the correct active pharmaceutical ingredients [9-11].

Irrational use of antimicrobial agents, easy availability of antimicrobial agents and use of sub-standard and counterfeit drugs play a major role in the emergence and spread of drug resistance among communities in resource-limited countries like Tanzania [12-15]. Also, implicated in the rise of resistant bacteria are the

*Author to whom correspondence may be addressed.

use of low-cost, less potent fluoroquinolones and the widespread addition of ciprofloxacin and other antibiotics to the feed of farm animals aimed at enhancing their productivity [11].

Ascertaining the quality of drug products involves the use of various procedures which include both biopharmaceutical and chemical assay techniques. Various methods have been reported for the chemical assay of ciprofloxacin tablets [16-18]. The choice of the method depends on local needs and resources. However, the disk diffusion test is still the most common test used for antimicrobial susceptibility testing.

METHODS AND MATERIALS

The study was conducted at Pharmaceutical Microbiology Laboratory, School of Pharmacy, Muhimbili University of Health and Allied Sciences (MUHAS). The study was carried out to assess the antibacterial effects of different brands of ciprofloxacin tablets available in Dar es Salaam drug outlets against three strains of bacteria, namely the Gram-positive *Staphylococcus aureus* and the Gram-negative *Pseudomonas aeruginosa* and *Escherichia coli*.

Sampling and sample size

Probability (cluster sampling) technique was employed for collection of ciprofloxacin (500 mg) tablet samples. The samples were randomly bought from pharmacies and medical stores located within Dar es Salaam City. A total of 9 different brands of ciprofloxacin each comprised of 15 tablets were used based on the following assumption: Each brand was purchased more than twice, but assayed separately for comparison purposes provided it was bought from distinct drug outlets/selling premises. Label and other relevant information (manufacturer's name, batch number, manufacture and expiry dates) displayed on the blisters or drugs' containers were recorded.

Chemical and biological materials

Reference strains of bacteria namely *S. aureus* (ATCC25923), *P. aeruginosa* (ATCC27853) and *E. coli* (ATCC25922), which are conserved in the Pharmaceutical Microbiology Laboratory, were employed for this purpose. All samples of ciprofloxacin tablets were obtained as described above, while positive control ciprofloxacin susceptibility discs (1 µg) and Mueller-Hinton agar were obtained from Medical Store Department (Dar es Salaam).

Antibiotics discs

Three tablets of each ciprofloxacin brand were crushed by mortar and dissolved in 0.1M HCl (to mimic the stomach conditions) and embedded in Whatman No. 1 filter papers from which disks of about 5 mm were cut out and employed for antimicrobial activity testing. The sensitivity discs were prepared as per the Clinical Laboratories Standards Institute (CLSI) guidelines [16] to contain the concentrations of 1 µg equivalent to the standards (commercial disc of 1 µg).

In order to get 1 µg from 500 mg of the antibiotic, each tablet was weighed and then crushed in a mortar. A given amount of the resultant powder was dissolved in 0.1M HCl of a given volume making a dilution of 1:20. The solution was transferred into a test tube and gently agitated for 24 h. At different time intervals (0, 15, 30, 45 and 90 min as well as after 24 h), a 20 µl aliquot was drawn and embedded onto 5 mm-diameter Whatman filter paper. An equal volume of 0.1M HCl was used as a negative control. Antibiotic susceptibility disc of ciprofloxacin (1 µg) was used as a positive control.

Antibacterial assay

Discrete colonies of the different identified isolates were inoculated into 5 ml of broth and incubated at 35°C for 24 h. The resultant microbial suspension was adjusted to match standard turbidity (McFarland 0.5M) prior to subjecting them to susceptibility tests as per CLSI recommendations [16]. The antibiotic susceptibility profiling of the bacterial strains on the test samples of ciprofloxacin tablets were evaluated using the Kirby-Bauer disc diffusion method [17, 19].

Each of the bacterial suspensions was aseptically inoculated into a different agar plate by the spread plate technique using sterile cotton wool. Using sterile forceps, each antibiotic disc (1 µg) was aseptically placed onto the agar plate. The discs were evenly distributed at 24 mm distance ensuring that they were at least 15 mm from the edge of the Petri dish. The agar plates were then incubated at 37°C for 24 h, before determination of the zones of inhibition (ZI). Interpretation of results was based on comparison of the ZI yielded against the control antibiotic discs and the test antibiotics by reference strains of bacteria as per the following ZI specifications: *S. aureus* (ATCC25923) ZI ranges 22-30 mm; *P. aeruginosa* (ATCC27853) with ZI ranges 25-33 mm; and *E. coli* (ATCC25922) with ZI ranges 30-40 mm [16, 17].

Statistical data analysis

All the above procedures were performed in triplicate, and the results are expressed as means. Statistical data analysis (means and variance for ZI) was carried out using the statistical package for social sciences (SPSS) computer software version 17 (Chicago, IL). Difference of ZI (antibacterial effects) among various groups (brands and batches) against each test bacterium and in comparison to the positive control were considered significant when $p < 0.05$.

RESULTS

A total of 9 different brands of ciprofloxacin tablets available in Dar es Salaam (Table 1) were assayed against 3 reference strains of bacteria viz. *S. aureus*, *P. aeruginosa* and *E. coli* yielding mean ZI of 23, 32 and 32 mm, respectively, which were within the acceptable limits. As shown in Figures 1-3, none of the brands attained the same antibacterial effects as that of positive control. Results of this study also indicate high variability of antibacterial effect of the tablets at time 0 especially for brand G (Figure 2) and brand I (Figure 3).

Table 1: Characteristics of the assayed samples of ciprofloxacin tablets

Brand	Origin	Batch	Date of manufacture	Date of expiry	Price/tablet (Tsh)	Weight (mg)
A	Germany	BXFN2	05/2010	05/2015	8,000	767
B	Switzerland	95226	11/2009	11/2014	2,300	750
C	Germany	2063	07/2010	06/2013	1,500	780
D	Greece	00171	04/2010	04/2013	1,100	769
E	India	45310	10/2010	10/2015	400	737
F	Korea	9004	11/2009	11/2012*	300	885
G	India	90382	05/2009	04/2012*	200	750
H	India	04140	04/2010	03/2014	150	803
I	Tanzania	10013	05/2010	04/2013	100	709

*Assay conducted before 2012.

Effects against *Staphylococcus aureus*

Brand A produced the largest ZI (18.47 + 0.15 mm) while brand I yielded the least ZI (15.2 + 0.34 mm). Moreover, the antibacterial effects exhibited by all brands depicted significant differences among them ($p = 0.001$; $F = 61.432$; $df = 8$). At 45 min, brand A exhibited the highest effect (ZI = 19.0 + 0.4 mm) while brand I and brand H with ZI = 15.83 + 0.15 mm were the least effective. The antibacterial effects were significantly different among the samples ($p = 0.002$; $F = 5.241$; $df = 8$). At 90 min, brand B (ZI = 20.07 + 0.404 mm) and brand H (ZI = 18.23 +

0.305 mm) were the most and least effective, respectively (p = 0.0001; F = 17.45; df = 8). Likewise, significant antibacterial effect differences were observed at 24 h. Brand A (ZI = 20.83 + 0.42 mm), and both brand H and brand G (both with ZI = 19.4 + 0.35 mm) being the most and least effective, respectively (p = 0.001; F = 13.34; df = 8). Most of the tested samples had less than the expected antibacterial effects against *S. aureus* (Figure 1).

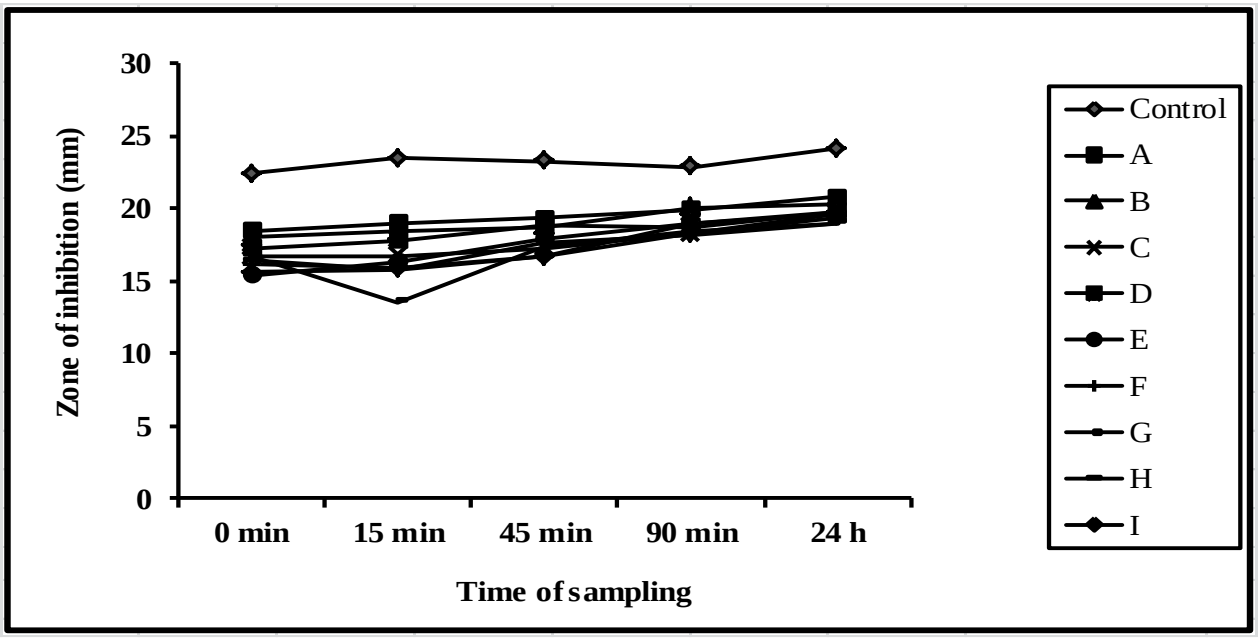

Figure 1. Comparative antibacterial effects of ciprofloxacin brands against *Staphylococcus aureus*.

Effects against *Pseudomonas aeruginosa*

Significant differences in antibacterial effects were observed among the brands with respect to this bacterium at 0 min (p = 0.190; df = 8; F = 1.612). However, the maximum antibacterial effect was exerted by brand A (ZI = 27.33 + 0.47 mm) and the least by brand G (16.04 + 13.8 mm). At 15 min interval, brand A and brand G exhibited the following patterns: ZI = 28.63 + 0.603 mm and 24.97 + 0.252 mm being the largest and smallest ZI, respectively. Statistically significant different antibacterial effects were observed among the brands (p = 0.0001; df = 8). At 45 min to 24 h intervals, brand A displayed the most potent antibacterial effects as shown in Figure 2. Moreover, significant differences of ZI were noted among the brands (p < 0.001). Only brands G and H could not attain the expected ZI at 24 h test as compared to the positive control (Figure 2).

Effects against *Escherichia coli*

Significant differences in antibacterial effects were observed among assayed brands against the *E. coli* at 0 min (p = 0.643; F = 0.758; df = 8). Brand C was the most effective with ZI = 29.033 + 0.25 mm and brand I the least with ZI = 19.93 + 0.153 mm (p = 0.0001; F = 64.191; df = 8). No significant different antibacterial effects were evident at 45 min (p = 0.288; F = 1.34; df = 7). But significant differences of antibacterial effects were observed at 90 min and 24 h (p = 0001) among the brands. Apparently, brands C (ZI = 32.10 + 0.26 mm) and A (ZI = 33.83 + 0.32 mm) were the most effective while brands H (ZI = 28.6 + 0.1 mm) and G (ZI = 30.4 + 0.36 mm) were the least effective at 90 min and 24 h intervals, respectively. The observed ZI for brands D, G, H and I were below the acceptable range (30-40 mm) at all the time intervals with exception of 24 h time point (Figure 3).

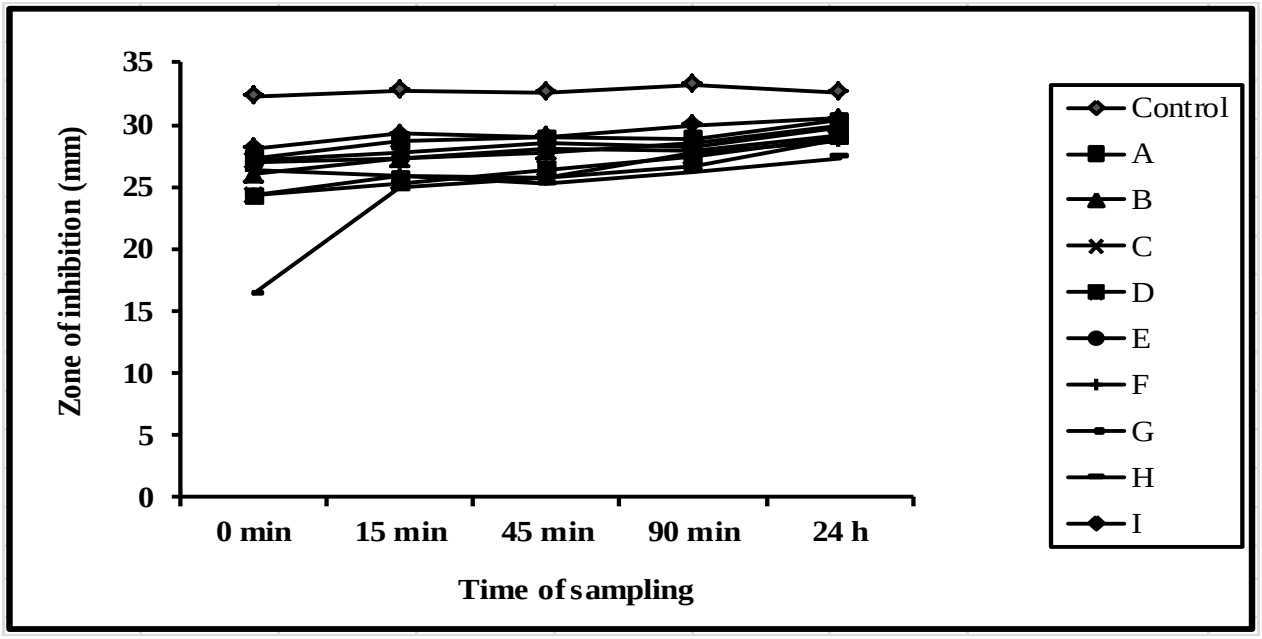

Figure 2. Comparative antibacterial effects of ciprofloxacin brands against *Pseudomonas aeruginosa*.

Overall comparison of antibacterial effects of test samples with control antibiotic

Student T-test revealed there were significant differences of antibacterial effects exerted by brand A against *P. aeruginosa* when compared to the control (30 mm) at all tested time intervals except at 15 min ($p = 0.059$; $t = -3.927$) and 24 h ($p = 0.469$; $t = 0.887$). At 45 min, no significant differences were observed between the positive control and brand A against *E. coli* ($p = 0.123$; $t = -2.580$). However, significant differences were observed throughout the tested time intervals in respect to the control against *S. aureus* ($p < 0.002$).

Brand B exhibited significant antibacterial differences against *P. aeruginosa* as compared to positive control with exception of the 24 h test interval ($p = 1.000$; $t = 0$). But significant differences were noted throughout the assay time on *E. coli* and *S. aureus* ($p < 0.004$). Brands C, D, E, F and H produced ZI that differed significantly from that of positive control on all test bacteria ($p < 0.05$). Brand G showed significant antibacterial differences against all test bacteria as compared to control with exception of *P. aeruginosa* at 0 min ($p = 0.231$; $t = -1.703$). Similarly, brand I showed significant antibacterial differences against all test bacteria as compared to control with exception to *E. coli* at 0 min ($p = 0.239$; $t = -1.657$).

Correlation between price and antibacterial effects of ciprofloxacin brands

Brand A was the most expensive while brand I was the cheapest. Of these 9 brands, 8 were imported from 5 different countries. The imported brands were the most expensive (Table 1). A positive correlation between the antibacterial effects and price was noted though statistically not significant ($R = 0.119$; $p = 0.290$). On the other hand, a negative correlation was observed between tablet weight and the price, but also was not statistically significant ($R = 0.034$; $p = 0.761$).

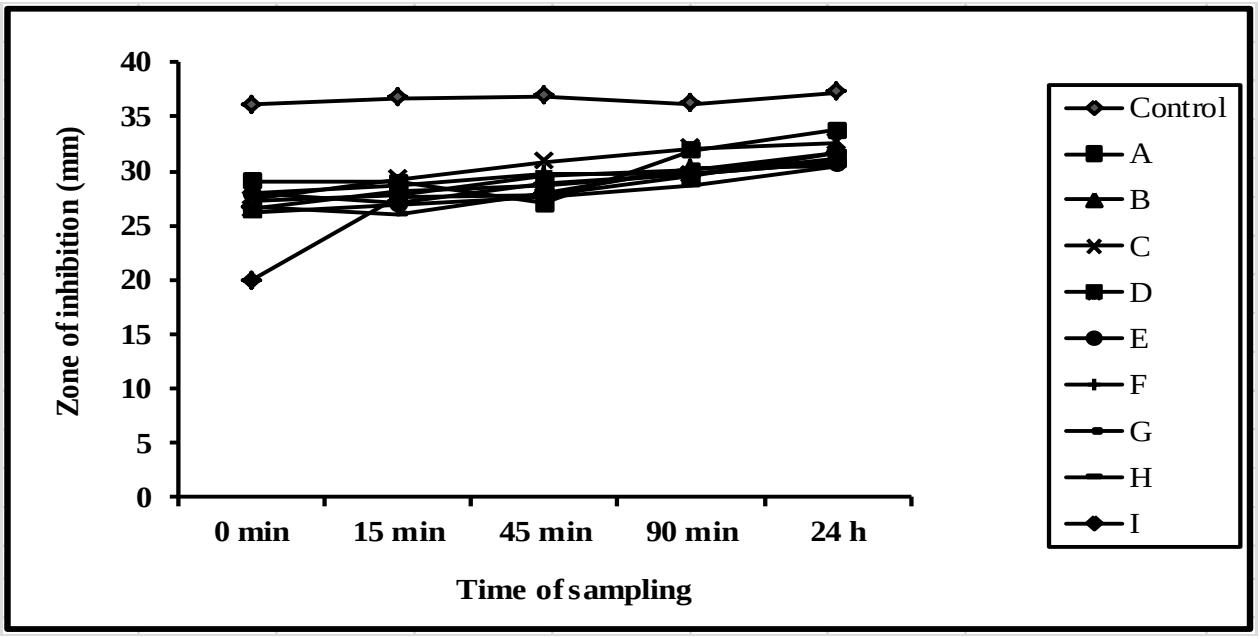

Figure 3. Comparative antibacterial effects of ciprofloxacin brands against *Escherichia coli*.

DISCUSSION

Sub-standard antimicrobial agents are a serious public health problem with demoralizing consequences on patients and advancement of antimicrobial resistance [20-24]. The Tanzania Foods and Drug Authority (TFDA) estimates that half of human drugs entering Tanzania are sub-standard or counterfeit [14]. The surveillance program conducted by TFDA indicated that only 3.7% of all tested drugs are sub-standard, half of which were counterfeit. However, TFDA claims that the rate of substandard drugs in Tanzania has fallen considerably from 13% a decade ago to currently 3.7% [15]. A recent study by the Confederation of Tanzania Industries (CTI) reveals that 60% of the medicines imported into the country are counterfeit and that 80% of the medicines used in the country are of foreign origin [13].

Counterfeit and sub-standard goods not only cripple legitimate local industry by unfair competition but local services firms and channel players also lose revenue while businesses waste time and money working with faulty and unsupported products [13]. The WHO also indicates that 700,000 Africans die annually from consuming fake anti-malarial or anti-tubercular drugs, most of which originate from China and India. Counterfeit drugs can also prejudice health by causing their users to develop a tolerance for the currently effective drugs and converting them to inefficacious antimicrobial agents [23, 25]. Counterfeiting also causes serious harm to the reputation of the genuine pharmaceutical manufacturers and makes them liable to any harm that may result from consumers ingesting counterfeit medicines [26]. Ciprofloxacin resistance is becoming one of the serious health concerns, particularly in resource-limited countries [25]. Many studies worldwide reported a clear upsurge in ciprofloxacin resistance [7, 8, 21-23]. This could partly be attributed to high prevalence of sub-standard drugs and/or irrational use of the same.

Generally, it is well known that reference bacterial strains like those employed in our study have no drug resistant traits. Hence, the antibacterial effects of the tested ciprofloxacin brands should ideally not vary significantly among them and/or as compared to the positive control. Therefore, the variability of antibacterial effects of the ciprofloxacin tablets revealed in this study are entirely due to intrinsic factors of each brand and not subject to method used, since previous studies had shown no differences with other methods like minimum inhibitory concentration [17, 18]. All brands were

relatively effective against *P. aeruginosa* yielding ZI within the acceptable range. Besides, all brands were as equally effective against *E. coli* as the control at 90 min. However, four brands (C, G, H and I) proved to be not very effective against *S. aureus*, producing ZI below the lower limits (22-30 mm). This may imply that ciprofloxacin may no longer be a drug of choice for *S. aureus* associated infections [19].

Due to budgetary constraints, Tanzania is unable to adequately control its lengthy and porous borders. Moreover, the available scarce resources are directed to other more stern problems: disease burden particularly HIV/AIDS, education and food security. Consequently, very little resources, if any at all, are allocated to the fight against importation/introduction of poor quality drugs. Our results indicate that 4 out of the 9 tested brands of ciprofloxacin (44.4%) consistently produced unexpectedly inferior antibacterial effects. The results show that poor quality drugs are a major public health concern more than they are actually perceived by national and local authorities [14, 22, 27]. Currently, no country in the world is free of counterfeited drugs, although South-east Asia and Africa are apparently the most affected [25].

Our results reveal immense price differences among the brands, and indicate a slight positive correlation between the price and effectiveness of the brands. This is very unfortunate for low-income earners who may not afford expensive and efficacious drugs. As previously indicated [23, 28], price monitoring may be a useful tool in detecting sub-standard or fake drugs simply because "the too-good-to be-true drug prices may well indicate poor quality products''. The surveillance for sub-standard drugs can therefore be more effective only if close follow-ups are made on the low-priced drugs. Sometimes the price of poor quality drugs overlaps greatly with that of good-quality drugs, suggesting that price is at most a weak signal of drug quality [24].

CONCLUSION

Brand A exhibited the most potent antibacterial effect (largest ZI) against the three test bacteria. Our study highlights a public health concern facing our country: high prevalence of poor quality antimicrobial agents that requires immediate intervention. A positive correlation between price of brands and antibacterial effect, which could serve as an indicator of drug quality present in our market, was revealed. We therefore recommend enforcement of post-market surveillance of medicines available in our market and more stern measures to prevent importation/entry of poor quality drugs. This may mitigate the fast growing pandemic of antimicrobial resistance to currently treatable microbial infections.

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