Zagazig J. Pharm. Sci. Jun, 2017 lSSN 2356_9786
Vol. 26, Issue 1, pp, 48-54 ISSN (on-line) 2356_9786

Evaluation of the potency of some antibiotic formulations in the Egyptian market

Hemat K. Abdullatif, Amira M. El-Ganiny, Afaf S. El-Adl*.
Department of Microbiology and Immunology, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt.
*Corresponding author e-mail: afafaladl@gmail.com

ABSTRACT

Interest in searching and developing new antimicrobial agents to combat microbial resistance has been growing recently. Therefore, a greater attention has been paid to both screening and evaluation methods of antibiotics activity. The present study aimed to evaluate the potency of some antibiotics containing pharmaceutical products of some Egyptian market companies using microbiological assay based on agar diffusion method and using standard strains in order to determine their therapeutic efficacy.

This antibiotics such as gentamicin, ciprofloxacin, doxycycline, amoxicillin and ceftriaxone were purchased from local pharmacies and evaluated in the current study.

The results of this study showed the relative potency of gentamicin was 41.4%-120% and 28%-41% for ciprofloxacin. While for doxycycline relative potency was 26%-72.6% and 16%-88% for Amoxicillin. As well as ceftriaxone potency was ranged between 48%-97.4%. One product of ceftriaxone, two products from gentamicin and two from amoxicillin were estimated to be within the acceptable range of bioequivalence (80%-120%), while the other products showed unacceptable relative potency. A complaint reporting system about quality and effectiveness problems needs to be considered as a priority source of such information to inform decision-makers.

Key words: antibiotices, evaluation, microbiological assay, agar diffusion method, potency

INTRODUCTION

Due to increasing microbial resistance problems to antimicrobial agents, the quantification of the actual concentration of active ingredient in antibiotic preparation is critical (**Balouiri *et al.*, 2016).** The potency of antibiotics can be determined by chemical or biological methods. These methods include microbiological assays, automated chemical assays (e.g. high performance liquid chromatography; HPLC), immunological assays (e.g. fluorescence immunoassay) and radioimmunoassay (**Dafale *et al.*, 2014; Dafale *et al.*, 2016).**

It is necessary to assay antimicrobial agents for determination of potency of antimicrobial therapy **(Pitkin and Martin-Mazuelos, 2007)**. Microbiological assay may be defined as qualitative or quantitative determination of any chemical compound with the use of microorganisms **(Kameshwar, 2017).** Microbiological assays are relatively as accurate as chemical methods. In addition it is simple, specific, inexpensive and convenient method **(Bekele and Gebeyehu, 2012).** Compared with biological assay methods using animals, microbiological techniques possess the advantages of minimal requirements of space, materials and time (**Kokare, 2016)**. Microbiological assay helps estimate active constituents, biological activity and monitor the stability of antibiotics. Any small change in the antibiotic molecule, which may not be detected by chemical methods, will be revealed by a change in antimicrobial activity **(Dafale *et al.*, 2014).**

Bacterial susceptibility to antibiotics can be done by a number of techniques, which include the disc or well diffusion method, the broth dilution assay, and the antimicrobial gradient method (E-tests) (**Balouiri *et al.*, 2016).** Quantification of antibiotic components by

chemical methods, such as HPLC and UV spectrophotometry, although precise, cannot provide a true indication of biological activity **(Salgado *et al.*, 2006).** Attempts to correlate antibiotic bioassay results with those from chemical methods have proved disappointing. Therefore, bioassays continue to play an essential role in quality control of antibiotic medicines **(Cazedey and Salgado, 2011)**

The agar diffusion method depends on antibiotic diffusion through a layer of solidified agar, in an extension that totally inhibits the growth of the microorganism in a zone around a reservoir containing antibiotic solution **(Lourenço and Pinto, 2009).** In this assay the inhibition zone size and the dose of the substance assayed are correlated. This is the most widely employed method to determine the potency of antibiotics. The microbiological assay of an antibiotic is based upon a comparison of the inhibition of growth of microorganisms under standardized conditions by measured concentrations of the antibiotics under examination (**Lourenco and Pinto, 2009).** The relationship between concentration logarithm of the antibiotic and the diameter of the zone of inhibition must be shown approximately rectilinear for the system used **(Zuluaga *et al*., 2009)**.

Due to high cost of exported antimicrobial drugs from multinational companies, this study was designed to determine the potency of some antibiotics found in the Egyptian market either from local or international companies.

Materials and methods:

Microbial strains:

Microbial strains were used in this study such as *Staphylococcus aureus* ATCC 6538 and *Pseudomonas aeruginosa* ATCC 9027 were obtained from the Egyptian Pharmaceutical Industries Company (EPICO), Egypt. While *Pseudomonas aeruginosa*PAO1 was obtained from Dr. Mohammed Yuossef at Microbiology Department, Mansoura University and *Micrococcus luteus* ATCC 9341 was obtained from Prof. Dr. Tarek El-Banna, Microbiology Department, Tanta University.

Antimicrobial agents:

Different dosage forms that contain the following antibiotics: gentamicin (Gen), ciprofloxacin (Cip), doxycycline (Dox), amoxicillin (Amx) and ceftriaxone (CTX) were purchased from local pharmacies and evaluated in the current study (**Table 1**). Also authentic powders of all tested antibiotics were obtained from National Organization for Drug Control and Research (NODCAR).

Staphylococcus aureus ATCC 6538 was used for assay of doxycycline**,** amoxicillin and ceftriaxone (**Mantio *et al.*, 2013)**. *Pseudomonas aeruginosa* ATCC 9027 was used for assay of gentamicin (**Eurofins Pharma Discovery Services, 2016)**. *Pseudomonas aeruginosa* PAO1 was used for assay of ciprofloxacin, while *Micrococcus luteus* ATCC 9341 was used for amoxicillin assay (**The Antimicrobial index Knowledgebase, TOKU-E).**

Antibiotic assay method:

Antibiotic assay was performed by agar-well diffusion method (according to **The International Pharmacopoeia-6th Edition** (**2016**). Petri dishes were filled to a depth of 4 mm with a culture medium (muller hinton agar) that has previously been inoculated with a suitable inoculum of a susceptible test organism (The final inoculum size was adjusted to 5 x 10^5 cfu/ml attained a turbidity of 0.5 McFarland standard). A 1.0 ml of the standard suspension of each test bacterial strain was seeded evenly on molten Mueller Hinton agar (Oxoid) of 48-50 °C and poured into plate. During the filling they should be placed on a flat surface so as to ensure that the layer of the medium will be of a uniform thickness. The plates were allowed to dry at room temperature. Subsequently, 10-mm diameter wells were bored in the agar and a 100 µl volume of different concentrations of antibiotics were transferred into the wells. Plates were

incubated at 37ºC for 24 h. Inhibition zone diameter (IZD) was measured to the nearest millimeter (mm). The arrangement on the plate were such that overlapping of zones is avoided.

Table 1: Antibiotic containing pharmaceutical products evaluated in this study

Antibiotic	**product code**	**Dosage form**	**Concentration** (mg)
Gentamicin	Gen1	Ampoules	80mg/2ml
	Gen2	Ampoules	80mg/2ml
	Gen3	Ampoules	40mg/ml
	Gen4	Ampoules	80mg/2ml
Ciprofloxacin	Cip 1	Tablets	250mg
	Cip 2	Tablets	500mg
	Cip 3	Tablets	500mg
	Cip 4	Tablets	500 mg
	Cip 5	Tablets	250 mg
Doxycycline	Dox 1	Capsule	100 mg
	Dox 2	Capsule	100 mg
	Dox 3	Capsule	100 mg
	Dox 4	Capsule	100 mg
Amoxycillin	Amx 1	Capsule	500 mg
	Amx 2	Capsule	500 mg
	Amx 3	Capsule	500 mg
	Amx 4	Vial	500 mg
	Amx 5	Capsule	500 mg
	Amx 6	Capsule	500 mg
Ceftriaxone	CTX 1	Vial	500 mg
	CTX 2	Vial	500 mg
	CTX 3	Vial	500 mg
	CTX 4	Vial	500 mg
	CTX 5	Vial	500 mg
	CTX 6	Vial	500 mg
	CTX 7	Vial	1gm

Solutions of the authentic antibiotics of known concentration and corresponding dilutions of the tested antibiotics were prepared in a sterile distilled water for soluble antibiotics or dimethyl sulfoxide (DMSO) (Sigma aldrish) for un-soluble antibiotics. To assess the assay validity, at least 3 different doses of the reference material were used together with an equal number of doses of the test substance having the same presumed activity as the solutions of the reference material. The dose levels used should be in geometric progression, for example, by preparing a series of dilutions in two folds.

The potency of different antibiotics was calculated according to the British Pharmacopea formulae (**BP, 2011**) as follow:

I= (log ratio of doses) = log 2 = 0.301

E= (effect due to dose) = 1/4 (S1 –S3 + T1-T3)

F= (effect due to test) = 1/3 (T1 -S1 + T2- S2 + T3 -S3)

b (slope) = E/I =E/0.301

M = F/b

Potency ratio T/S = antilog of M

Results and Discussion

Correlation between concentration of antibiotics and inhibition zone diameter

The diameter of inhibition zones of test antibiotics using standard bacterial strains were measured. All cases exhibited

a linear relationship between the concentration of the antibiotic and inhibition zone diameter.

The potency was calculated according to British Pharmacopeia (BP) formulae and results are shown in **Table 2.**

Table (2): Potency of different antibiotics from different companies relative to their authentic reference

Product code	Dosage form	Labeled conc.	Calculated potency	Real conc.	Reference organism
Gen1	Ampoules	40mg/ml	115%	46mg/ml	***Pseudomonas aeruginosa*** **ATCC 9027**
Gen2	Ampoules	40mg/ml	49.5%	19.8 mg/ml	
Gen3	Ampoules	40mg/ml	120%	48 mg/ml	
Gen4	Ampoules	40mg/ml	41.4%	16.5 mg/ml	
Cip 1	Tablets	250mg	28%	70 mg	***Pseudomonas aeruginosa*** **PAO1**
Cip 2	Tablets	500mg	39%	195 mg	
Cip 3	Tablets	500mg	25%	125 mg	
Cip 4	Tablets	500mg	26%	130 mg	
Cip 5	Tablets	250mg	41%	102.5 mg	
Dox 1	Capsule	100mg	26%	26 mg	***Staphylococcus aureus*** **ATCC 6538**
Dox 2	Capsule	100mg	72.6%	72.6 mg	
Dox 3	Capsule	100mg	27%	27 mg	
Dox 4	Capsule	100mg	70%	70 mg	
Amx 1	Capsule	500mg	68%	340 mg	
Amx 2	Capsule	500mg	70%	350 mg	***Staphylococcus aureus*** **ATCC 6538**
Amx 3	Capsule	500mg	83%	415 mg	
Amx4	Vial	500mg	100%	500 mg	
Amx 5	Capsule	500mg	70%	350 mg	
Amx 6	Capsule	500mg	16%	80 mg	
Amx 1	Capsule	500mg	44.3%	221.5 mg	
Amx 2	Capsule	500mg	56%	280 mg	***Micrococcus luteus*** **ATCC 9341**
Amx 3	Capsule	500mg	66 %	330 mg	
Amx4	Vial	500mg	88%	440 mg	
Amx 5	Capsule	500mg	56%	280 mg	
Amx 6	Capsule	500mg	15.8%	79 mg	
CTX 1	Vial	500mg	48%	240 mg	
CTX 2	Vial	500 mg	65%	325 mg	***Staphylococcus aureus*** **ATCC 6538**
CTX 3	Vial	500mg	97.4%	487 mg	
CTX 4	Vial	500mg	74.6%	373 mg	
CTX 5	Vial	500mg	63.6%	318 mg	
CTX 6	Vial	500mg	96%	480 mg	
CTX 7	Vial	1gm	49.7%	497 mg	

Percentage of potency for gentamicin were 115% , 49.5%, 120% and 41.4% for Gen 1, 2, 3, 4 respectively.The results obtained in this study are consistent with the reservations about gentamicin products reported in quality monitoring surveys which is almost 103.50% (**Kumar and Ramya, 2012)** for Gen 1 and Gen3 but disagree with Gen 2 and Gen 4.

For ciprofloxacin, potency was recorded as 28%, 39%, 25%, 26% and 41% for Cip1, Cip2, Cip 3 and Cip 4, respectively. Our results were lower than that reported by **Cazedey and Salgado (2011)** with orbifloxacin where the activity ranged from 99.18 - 101.84% . The results of this study revealed that the equivalence and potency of generic

products of doxycycline capsules were 26%, 72.6%, 27% and 70% of the labelled activity for Dox 1, Dox 2, Dox 3 and Dox 4, respectively.

In this study , the obtained result revealed that the potency of ceftriaxone in the tested product were 48% ,65% ,97.4% ,74.6% ,63.6 % ,96% and 49.7% for CTX 1, 2, 3, 4, 5, 6 and 7 , respectively. The result agreed with that reported by **(Idries and Ibrahim , 2014)** that potency was 98.6% for CTX 3, CTX 6 while it was higher than that reported for CTX 1, 2, 4, 5 and 7 the tested products were not equivalent to the authentic reference product under testing.

Although antibiotics are used to treat various diseases, nowadays they are becoming a major problem in health sector as microbial resistance against these drugs has been emerged. Among the reasons for microbial resistance are, insufficient access to effectual drugs and sometimes drugs of doubtful quality and overall poverty may play role in the development of antimicrobial resistance **(Modak *et al.*, 2013)**.

The equivalency of pharmaceutical products is an essential and basic criterion for proving the quality of any product **(Bajaj *et al.*, 2012).** In general, there are different methods that can be used to evaluate the drugs potency; including the *in vitro* bioassays **(Pegler, 2010).** A3 x 3 microbiological assay was proposed for determining the concentrations in pharmaceutical dosage forms according to British pharmacopeia.

Our results show that the potency of Amoxicillin capsules generic products on *Staphylococcus aureus* ATCC 6538 were 68%, 70%, 83%, 100%, 70% and 16% for Amx 1, 2, 3, 4, 5, 6 respectively. While, the amount of ampicillin calculated was within the range between 90 and 115%, recommended by the Brazilian and United States pharmacopeias **(Tótoli and Salgado, 2013)** agree with Amx 3 and Amx 4 only

In this study, Amoxicillin capsules also assayed by using *Micrococcus luteus* ATCC 9341. The obtained data exhibited that the potency of amoxicillin were 44.3%, 56%, 66%, 88%, 56%, 15.8% for Amox 1, 2, 3, 4, 5 and 6, respectively. These data indicate the sensitivity of *Micrococcus luteus* ATCC9341 as a reference organism.

Accordingly, it is not anticipated to show a similar therapeutic profile and effectiveness. Considering the critical importance of anti-microbial agents to public health, it has now become essential to investigate the assumption about the differences in therapeutic equivalency between the products available on the market **(Idries and Ibrahim, 2014)**.

Our study indicates that using a well-designed microbiological bioassay should help medicines regularity authorities to perform such re-evaluations to check the quality of different products. In general, this study also highlighted the importance of expanding post-marketing surveillance systems to check both the quality and efficacy of antibiotics using microbiological assays, bioassays, bioequivalence studies and dissolution test as routine tests.

In conclusion, this simple method of analysis is very important for any regulatory authority to be considered a routine process. The microbiological method provides a good tool for accurate determination of the potency of antibiotics. In addition, a complaint reporting system about quality problems needs to be considered as a priority source of such information to inform the decision makers.

References

Bajaj, S. , Singla, D. , Sakhuja, N. (2012): Stability Testing of Pharmaceutical Products. *Journal of Applied Pharmaceutical Science,* 2 (3): 129 - 138

Balouiri, M., Sadiki, M., Ibnsouda, S.K. (2016): Methods for *in*

vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, 6(2):71-79

Bekele, L.K., Gebeyehu**,** G.G. (2012): Application of different analytical techniques and microbiological assays for the analysis of macrolide antibiotics from pharmaceutical dosage forms and biological Matrices: A review. *ISRN Analytical Chemistry*, 2012: 17 pages.

British pharmacopeia (2011) The Stationary Office, London, pp. A348−A355

Cazedey, E. C. L., Salgado , H. R. N. (2011): Development and validation of a microbiological agar assay for determination of orbifloxacin in pharmaceutical Preparations. *Pharmaceutics,* 3(3): 572-581

Dafale, N.A., Semwal, U.P., Agarwal , P.K. (2014): Development and validation of microbial bioassay for quantification of levofloxacin in pharmaceutical preparations. *Journal .Pharm. Anal*., 5: 18–26

Dafale, N.A. , Semwal , U.P. , Rajput, R.K. , Singh, G.N. (2016): Selection of appropriate analytical tools to determine the potency and bioactivity of antibiotics and antibiotic resistance. *Journal of Pharmaceutical Analysis*, 6(4): 207-213.

Eurofins Pharma Discovery Services, Assay Catalog, 2016 Jul 26

European Pharmacopoeia. 2005. *Method of Analysis*, 5th Ed Vol. 22 (2), 74-79. Strasbourg, France: Council of Europe.

Idries, A.M., Ibrahim , K.E. (2014): Microbiological Assay of two selected products of cephtriaxone powder for injection from pharmaceuticals market in Sudan. *Sudan Journal of Medical Sciences*, 9(4): 251-258.

Kameshwar, A.K.S (2017): Qualitative and quantitative methods for isolation and characterization of lignin-modifying enzymes secreted by microorganisms, *BioEnergy Research*, 10 (1): 248–266

Kokare, C. R. (2008). Pharmaceutical microbiology-principles and applications. In "Basic Microbiology - For Nursing and Health Science". First Edition. By *Nirali Prakashan. Pune, 6.*

Kumar, G.A., Ramya, V. (2012): Quantification of gentamicin by microbial assay technique and reverse phase HPLC. *European Journal of Experimental Biology*, 2(6) : 2083-2089

Lourenço, F.R., Pinto, T.D.J. (2009): Comparison of three experimental designs employed in gentamicin microbiological assay through agar diffusion. *Brazilian Journal of Pharmaceutical Sciences*, 45(3): 595-566

Mantio, M.L, Agarrayua, D.A., Machado, J.C, Schmidt, C.A. (2013): A fully validated microbiological assay to evaluate the potency of ceftriaxone sodium. *Brazilian Journal of Pharmaceutical Sciences*, 49(4): 753-762.

Modak, B., Shahinur Rahman, M., Gazi, M.S., Aftab Uddin, M., Rahman, T. (2013): Antimicrobial potency assay of common antibiotics collected from different drug stores in Dhaka Metropolis. *Stamford Journal of Microbiology*, 3(1): 26-29

Pegler, S. (2010). Evaluating the safety and effectiveness of new drugs. *Obstetrics & Gynecology*, 82(1): 53-57

Pitkin, D. H., Martin-Mazuelos, E. (2007). 14 Bioassay Methods for Antimicrobial and Antifungal Agents. *Antimicrobial Susceptibility Testing Protocols*, 313- 336.

Salgado, H.R.N., Lopes, C.C.G.O., Lucchesi, M.B.B. (2006): Microbiological assay for gatifloxacin in pharmaceutical formulations. *Journal of*

pharmaceutical and biomedical analysis, 40 (2): 443 – 446

The Antimicrobial index Knowledge base – TOKU-E) http://antibiotics.toku-e.com/microorganism_

The International Pharmacopoeia (2016). Quality specifications for pharmaceutical substances and dosage forms together with supporting general methods for analysis WHO -6th Edition http://apps.who.int/phint/pdf

The United States Pharmacopeial Convention (2010) Pharmacopeial Forum, 36(6): 1-35

Tótoli, E. G., Salgado, H. R. N. (2013): Development and validation of a rapid turbidimetric assay to determine the potency of ampicillin sodium in powder for injectable solution. *Analytical Methods*, *5*(21), 5923-5928.

Zuluaga, A.F., Agudelo, M., Rodriguez, C. A., Vesga, O. (2009): Application of microbiological assay to determine pharmaceutical equivalence of generic intravenous antibiotics. *BMC Clinical Pharmacology*, 9(1), 1.

تقييم فاعليه بعض المستحضرات التي تحتوي علي المضادات الحيويه في السوق المصري

ا د /همت كمال عبداللطيف- د/اميره محمد الجنايني – عفاف صبحي العدل
قسم الميكروبيولوجي والمناعه-كليه الصيدله جامعة الزقازيق

في السنوات الاخيره زاد الشغف في مجال البحث لتطوير المضادات الحيوية للتغلب علي مقاومه البكتريا لها لذلك وجب تقييم ومعايرة فاعلية وتأثير المضادات الحيويه الموجودة في الاسواق للتاكد من فاعليتها.
فاعلية المضادات الحيوية يمكن ان تقاس بعدة طرق منها مقارنة قدره المضاد الحيوي علي منع نمو البكتريا بتركيز معين من المضاد الحيوي الحقيقي الاصلي و المضاد الحيوي المراد تقييمه
الهدف من هذه الدراسه هو تقييم ومعايرة المواد المضاده للبكتريا من شركات مختلفه في السوق المصري بطريقةالانتشار في الاجار باستخدام عزلات اساسيه ذات معايير لتعيين فاعليتهم العلاجيه
تراوحت فاعلية المضادات الحيوية التي تحتوي علي الجينتاميسن ٤١,٤%-١٢٠% وفاعليه السيبروفلوكساسين ٢٨%-٤١% اما الدوكساسيكلن فوجدت ٢٦%-٧٢,٦% فاعلية الاموكساسيلن ١٦%-٨٨% اما السيفترياكسون كانت بين ٤٨%-٩٧,٤%
و اجمالا هناك منتج واحد فقط من السيفترياكسون و اثنان من الجينتاميسن و اثنان من الاموكساسيلن كانت ذات فاعليه مقبولة (٨٠%-١٢٠%) اما باقي المنتجات فكانت فاعليتها غير مقبولة.