



Screening of Antimicrobial Activity from Various Pharmaceutical Companies in Iraq

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Abstract

An antimicrobial is an agent that kills microorganisms or stops their growth. Antimicrobial can be classified according to their function, it may be a microbicidal which is an agent that kill microbes, while those that only prevent and stops their growth are named biostatic. Antimicrobial resistance (AMR) is the ability of a microbe to resist the effects of antimicrobial medication that once could successfully treat the microbe by killing or stop its growth. So with the resistance to the antibiotics becoming more and more common and worldwide resulting in need for screening the antimicrobial activity. So in this study we used an evaluation method which is used in many laboratories to screen the activity of antimicrobial agents obtained from a local company and a foreign company on standard microorganism which includes the following (*Escherichia coli*, *staphylococcus aurous*, *Pseudomonas aeruginosa*, *Candida albicans*) and pathogenic bacteria includes (*Escherichia coli*, *staphylococcus aurous*) which obtained from renal infections and tissue burns respectively. In conclusion the results show that the antibiotic manufactured in the local company Al-Razi in Iraq gave better effectiveness than other foreign companies and the reason for this difference may due to the active chemicals used in manufacturing the antibiotic or due to the manufacturing method.

1. Introduction

Antimicrobial is an agent that kills microorganisms or stops their growth. Antimicrobial agents can be classified in many ways, founded on four simple features, these features include antibacterial activity, class of microorganism, time- or concentration-dependent activity and bacteriostatic or bactericidal activity. Mechanisms of action fall into four categories: damage to cell membrane function, inhibition of nucleic acid synthesis or function, inhibition of cell wall synthesis, and inhibition of protein synthesis [1, 2]. Multiple drug resistance has become one of the biggest threats to global health all around the world, many common infections are becoming resistant to the antimicrobial medicines used to treat them, resulting in longer illnesses and more deaths. Today, one of the major challenges facing Iraq is the multidrug resistance especially in the military Injuries The most frequently identified resistant strains of bacteria are *Staphylococcus aurous* [3]. Evaluation is a laboratory method

used to screen the in vitro antimicrobial activity of a drugs, the evaluation of drug is essential because there may be biochemical variations in the drug. There may be deterioration due to storage substitution may be present as a result of carelessness [4]. The aim of the drug evaluation is to identify and determine the quality of the drug and detecting the nature of adulteration. Two of the evaluation methods used in this study:

1. Agar disk diffusion method: this method was developed in 1940 [5] it is the authorized method used in many microbiology laboratories for repetitive testing of antimicrobial susceptibility. Currently, several recognized and official standards are published by the Clinical and Laboratory Standards Institute (CLSI) for bacteria and yeasts testing. In this famous procedure, plates with agar are inoculated with a standardized inoculum of the test microorganism. Then, a filter paper in discs' form containing the compound used for testing in a wanted concentration, are placed on the agar surface. The Petri dishes must be incubated under an appropriate condition. Generally, the antimicrobial agent spreads into the agar and stops the growing of the test microorganism and then the diameters of inhibition zones are measured, the MIC can be calculated by matching the inhibition zones with stored algorithms [6].
2. Agar well diffusion method: This method is widely used to esteem the activity of antimicrobial, plants or microbial extracts [7, 8]. Likewise, to the technique of disk-diffusion method, the surface of the agar in plate was inoculated by spreading a volume of the microbial inoculum concluded the whole agar surface. Then, a hole with a diameter of 6 to 8 mm is cut aseptically with a sterile sterilized pasture pipette, then a volume (20–100 μ L) of the extract solution or antimicrobial agent with wanted concentration is introduced into the well [6]. Experimental Procedure

2. Experimental Procedure

1. Mueller Hinton agar is a medium that allows a better diffusion of the antibiotics than other media which leads to a proper inhibition zone [9] agar media was prepared for bacterial cultures: medium named Muller Hinton agar was prepared based on CLSI standard procedure [10] it's done by melting 38g of the agar in 1000 ml sterile distal water, heat until start boiling to melt the medium totally, then its Sterilized by autoclaving at (15lbs) pressure and 121°C for 20min, then the mixture is mixed well before poring. After that the media was poured into disposable Petri dishes in a depth of 3 to 4mm. After solidification, all the plates with medium were saved at 4°C till use.
2. Procedure for Disc diffusion method: By using cotton swab, a touch of bacterial in (normal saline) this suspension is compared with a McFarland tube It is used to check and correct the densities of bacterial suspension that used for the experiment. (0.5) McFarland turbidity standard provides an optical density comparable to the density of a bacterial suspension with a 1.5×10^8 colony forming units which is the standard used in the microbiological laboratories [11]. Then by cotton swab transfer the bacteria to the medium and by using the streaked method 3 times by circling the plate about 60° between streaking. To confirm smooth distribution of the inoculums, the inoculated plates were kept at 25°C for ten min. to let absorption of extra moisture. Then, put the disc (antibiotic or antifungal of each company) on the inoculate Petri dishes, and incubate it from 18_ 24 hrs [12]. After that, an inhibition zones were appeared and measured using ruler to determination their diameters in millimetres and compare the diameters with standards in CLSI.
3. Procedure for Well diffusion method: By using a cotton swab, a drop of bacteria in (normal saline) that was compared with a McFarland tube (0.5) to insure a proper concentration of bacteria in the tube. Then transferred to the medium and using the streaked method. The inoculated plates were kept at room temperature for ten min. to allow absorption of extra moisture. Then, using sterilized pasture pipette for making which were then filled with 50 μ l of the diluted (antibiotic or antifungal). Then the plates were incubated at 37°C for 18-24 hrs. [12]. After incubation, a clear zone was appeared and measured using ruler for determination their diameters in millimetres, and the results were compared with the standards in CLSI [10].

3. Results and Discussion

3.1. Antimicrobial Susceptibility Using Disk Diffusion Method

Antibiotic disk on ATCC bacteria: This standard method (disk diffusion method) was used to define the susceptibility of the ATCC bacteria to the 6 different type of local antibiotic disks and foreign antibiotic disks in

order to confirm their sensitivity and resistant as shown in table 1. Both local and foreign antibiotic disk gave same result except for (Cefoxitin) which the bacteria was sensitive for Al-Razi disk and resist for foreign disk.

Table (1). Antibiotic susceptibility of Al-Razi and Bioanalysis antibiotic disk on ATCC bacteria.

	AK Razi Bio	AK Bio	GEN Razi Bio	GEN Bio	CTR Razi Bio	CTR Bio	AMC Razi Bio	AMC Bio	CX Razi Bio	CX Bio	TE Razi Bio	TE Bio
E.coli	S	S	S	S	R	R	S	R	R	R	S	S
S.aureus	S	S	S	S	R	R	S	R	R	R	S	S
P.aeruginosa	S	S	S	S	S	S	S	R	R	R	S	S

Comparison of efficacy between Al-Razi and Bioanalysis discs on ATCC bacteria: This result confirm by CLSI standard. In contrast the result of Al-Razi center and Bioanalysis show some differences between them. Al-razi disk gave better sensitivity for (Cefoxitin) than the foreign disk which the three ATCC bacteria (*E. coli*, *S. aureus*, *P. aeruginosa*) were sensitive for Al-Razi compare to bioanalysis disk. it may be related to quality insurances for each one and the rules of manufacturing.

Antibiotic disk on pathogenic bacteria: The standard method which is disk diffusion method was used to determine the susceptibility of the pathogenic bacteria to the 6 different type of local antibiotic disks and foreign antibiotic disks in order to confirm their sensitivity and resistant as shown in Table (2). Both local and foreign antibiotic disk gave same result except for (Tetracycline) which the bacteria was sensitive for Al-Razi disk and resist for foreign disk.

Table (2). Antibiotic susceptibility of Al-Razi and Bioanalysis antibiotic disk on pathogenic bacteria.

Bacteria	AK Razi Bio	AK Bio	GEN Razi Bio	GEN Bio	CTR Razi Bio	CTR Bio	AMC Razi Bio	AMC Bio	CX Razi Bio	CX Bio	TE Razi Bio	TE Bio
<i>E.coli</i>	S	S	S	S	R	R	R	R	R	R	S	R
<i>S.aureus</i>	S	S	S	S	S	S	R	R	R	R	S	S

Comparison of efficacy between Al-Razi and Bioanalysis discs on pathogenic bacteria: the result of Al-Razi and Bioanalysis shows contradiction only with tetracycline which the disk from Al-Razi gave a result of sensitivity in opposite to bioanalysis disk on bacteria *E. coli*, it may be because the difference Nature of pathogenic isolation to which the experiment was applied.

Antifungal disk on ATCC fungus: The standard method (disk diffusion method) was used to determine the susceptibility of the fungus to the 2 different type of local antifungal disks and foreign antifungal disks in order to confirm their sensitivity and resistant as shown in Table (3). Our result shows that the (*C. albicans*) is resist to the both companies.

Table (3). Antifungal susceptibility of Al-Razi and Bioanalysis antifungal disk on ATCC fungus.

Fungi	KT Razi Bio	KT Bio	CC Razi Bio	CC Bio
<i>C. albicans</i>	R	R	R	R

Comparison of efficacy between Al-Razi and Bionalysis discs on ATCC fungus: This result Confirmed by CLSI standard. the *C. albicans* gave the same result (resist) to both Al-Razi and Bioanalysis discs.

Antimicrobial susceptibility using well diffusion method: Antibiotic vials on ATCC bacteria: The well diffusion method was used to determine the susceptibility of the ATCC bacteria to the 6 different type of foreign antibiotic vials (Equal to concentration of discs) in order to confirm their sensitivity and resistant as shown in Table (4).

Table (4). Antibiotic susceptibility of foreign antibiotic vials on ATCC bacteria.

Bacteria	AK foreign	GEN foreign	CTR foreign	AMC foreign	CX foreign	TE foreign
<i>E.coli</i>	S	S	R	R	R	S
<i>S.aureus</i>	S	S	R	R	R	S
<i>P.aeruginosa</i>	S	S	R	R	S	S

Comparison of efficacy between local and foreign antibiotics on ATCC bacteria using disc and well diffusion methods: the results showed The extent variation of different antibiotic from different companies, and the reason of these variance may be due to Differences in manufacturing in terms of the use of active substances or materials to help increase efficiency since the bacteria is ATCC. Cefoxitin from vials gave better effect compare to Al-razi and Bioanalysis for *P. aeruginosa*. although it was prepared as same concentration as the disk, it may be because of manufacturing of the substance. As for Amoxiclav, all three bacteria were sensitive to the disk from Al-razi compared to bioanalysis and vials.

Antibiotic vials on pathogenic bacteria: The well diffusion method was used to determine the susceptibility of the pathogenic bacteria to the 6 different type of foreign antibiotic vials (Equal to concentration of discs) in order to confirm their sensitivity and resistant as shown in Table (5).

Table (5). Antibiotic susceptibility of foreign antibiotic vials on pathogenic bacteria.

Bacteria	AK foreign	GEN foreign	CTR foreign	AMC foreign	CX foreign	TE foreign
<i>E.coli</i>	S	S	S	S	S	S
<i>S.aureus</i>	S	S	S	S	S	S

Comparison of efficacy between local and foreign antibiotics on pathogenic bacteria using disc and well diffusion methods: the result shows the difference Effectiveness between the antibiotics obtained from different companies, the vials gave better effect compared to the disks from Al-Razi and bioanalysis. This diversity may be due to the different nature of the isolation as it varies from region to region the antibiotic will have a different effectiveness on the pathogenic isolation.

Antifungal powder on ATCC fungus: Disk diffusion method was used to determine the susceptibility of the fungus to the 2 different type of foreign antifungal disks in order to confirm their sensitivity and resistant as shown in Table (6).

Table (6). Antifungal susceptibility of foreign antifungal powder.

Fungi	KT foreign	CC foreign
<i>C. albicans</i>	R	R

Comparison of efficacy between local and foreign Antifungal on ATCC fungus using disc and well diffusion methods: The *C. albicans* shows (resist) to both local, bio and vials antifungal.

4. Conclusions

Depending on the method used in evaluation of different antibiotic discs the result shows that the Al- Razi discs from Iraq have higher effectiveness than the bioanalysis discs from Jordan. We use only two methods to evaluation of quality for antibiotic between difference companies, it's not qualify to evaluation we need more different method to confirm the estimation.

References

- [1] U. Theuretzbacher, K. Bush, and S. Harbarth " Critical analysis of antibacterial agents in clinical development". *Nature Reviews Microbiology*, vol., 18, p. 1-3, 2020.
- [2] H. Ullah, and S. Ali, "Classification of Anti-Bacterial Agents and Their Functions", *In "Antibacterial Agents"* R. Kumavath. London, United Kingdom, 2017.
- [3] R.T. David, K.M. Clinton, A.L. Bradley, G. Anuradha, M. Katrin, M.B. Dana, L.P. Joseph and M. Jay "After the Battlefield: Infectious Complications among Wounded Warriors in the Trauma Infectious Disease Outcomes Study". *Military Medicine*. vol. 184, p. 18–25, 2019.
- [4] Y. Wang, J. F. Smith, M. M. Araya, K.-H. Liao and B. Gorovits "Development of a Highly Specific Anti-Drug Antibody Assay in Support of a Nanoparticle-based Therapeutic". *American Association of Pharmaceutical Scientists*, vol.22, no. 4, p. 81-94, 2020.
- [5] B. Mounyr and I.B. Saad, "Methods for in vitro evaluating antimicrobial activity: A review" *Journal of Pharmaceutical Analysis*, vol .6, no.2, p. 71-79, 2016.
- [6] R.M. Humphries, J. Ambler, S.L. Mitchell, M. Castanheira, T. Dingle, J.A. Hindler, L. Koethand and K. Sei, "CLSI Methods Development and Standardization Working Group Best Practices for Evaluation of Antimicrobial Susceptibility Tests", *Journal of Clinical Microbiology*. vol. 56 no. 4, p. e01934-17, 2018.
- [7] A. Alastruey-Izquierdo, MS. Melhem, LX. Bonfietti, JL. Rodriguez-Tudela, " Susceptibility test for fungi: Clinical and laboratorial correlations in medical mycology " *Revista do Instituto de Medicina Tropical de São Paulo*. vol.57, no.19, p.57-64, 2015.
- [8] S. Manandhar, S. Luitel, R.K. Dahal, " In Vitro Antimicrobial Activity of Some Medicinal Plants against Human Pathogenic Bacteria". *Journal of Tropical Medicine*, vol. 2, p.1-5, 2019.
- [9] N.S. Mohamed, H. A.Walaa ,and B. M. Wafaa "Evaluation of antibiotic susceptibility test results: how guilty a laboratory could be?" *Journal of the Egyptian Public Health Association*, vol. 94, no.4, 2019.
- [10] Clinical and Laboratory Standards Institute. Approved Standard. Performance standards for antimicrobial susceptibility testing, 32nd, 2022.
- [11] A. S. Fernanda, G. D. Vanessa and B. B. Thiago, "Use of McFarland Standards and Spectrophotometry for *Yarrowia Lipolytica* QU69 cell counting." *International Journal of Agriculture Environment and Biotechnology*, vol. 5, no. 4, pp. 1089-1091, 2020.
- [12] M. Balouiri, M. Sadiki and S.K. Ibsouda, "Methods for in vitro evaluating antimicrobial activity: A review". *Journal of Pharmaceutical Analysis*, vol. 2, p.71-79, 2016.