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Research Article

Direct Solid Disc as a Novel antibacterial testing method

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ABSTRACT

The most known and applied antibacterial testing methods are diffusion, dilution, and autobiography. These methods include many specific steps to screen the ability of tested agent to resist definite bacteria with several advantages and disadvantages.

The new approached method (Direct Solid Disc) eliminated many detailed steps and gave direct and accurate results than other manual techniques. This method was applied with several organic materials (Chloroxylenol, Ethylene diaminetetraaceticacid(EDTA), Pentachlorophenol, Vitamin B1, 5-Sulphasalicylic acid, and Ethylene diaminetetraacetic acid disodium salt) and the obtained results were compared with spotting diffusion results. By applying this approached method, many synthesized solid chemicals can be tested directly without thinking of solubility problems and limitations.

Keywords: Direct Solid Disc, antibacterial method, Chloroxylenol, Ethylene diaminetetraacetic acid(EDTA), Pentachlorophenol, Vitamin B1, 5-Sulphasalicylic acid, and Ethylene diaminetetraacetic acid disodium salt.

INTRODUCTION

Antibacterial testing methods are important techniques to investigate the ability of specific agent (metal, organo-metallic complex, organic derivative, alloy, polymer, extract …etc.) to resist characteristic bacterium species for particular disease. Microbiologists, chemists, or other scientific researchers (interested in this field) applied (disc, cylinder, and hole-plate) diffusion or (agar dilution and tube) dilution procedure as a manual mode beside (contact, immersion, and direct) autobiography method [1-5].

Both diffusion and autobiography methods are considered as qualitative screening test while the quantitative test can be done with dilution procedure [6]. The choice of a nominated procedure depends on its advantages and disadvantages such as time-saving, detection accuracy, sensitivity degree, flexibility, and financial implications.

These in vitro testing methods are facing a serious problem mainly depending on the solubility of the tested reagent and resulting a clear solution. The most used solvents for these tests were dimethyl sulfoxide (DMSO), dimethyl formamide (DMF),ethanol, methanol, petroleum ether, dichloromethane, chloroform, acetone, sterile water, …etc. especially with plant extraction[4, 7-12].

The choice of the solvent limited with specific consideration such as elimination of any inhibition effect on the microorganism under test, its toxicity, cost, increasing solubility with heat influence, …etc. These limitations promoted many promising antibacterial agent to be repealed from susceptibility screening of different bacterial strains. As a result of these limitation, we do think that antibacterial agent, that may be converted to antibacterial drug, must to be directly practiced to qualify its action upon bacteria without carrier.

The aim of this work was directed to test a new approach of antibacterial screening methods of different compounds by Direct Solid Disc and comparing it with standard methods.

EXPERIMENTAL SECTION

Materials:

Chloroxylenol, Ethylene diaminetetraacetic acid(EDTA), Pentachlorophenol, Vitamin B1, 5-Sulphasalicylic acid, potassium bromide were from BDH. Dimethyl sulfoxide was from Merck. Ethylene diaminetetraacetic acid disodium salt (EDTA-disodium salt) was from Hopin& Williams. Sterile water manufactured by Parenteral Drugs (India) limited [Bath no. 3103, Mfg. Feb. 13, Exp. Jan. 16] was registered by Iraqi Ministry of Health with number 405 in 12/01/1997. All tested compounds were used without further purification.

Methods:

Standard antibacterial methods:

These antibacterial procedures were performed according to the documented scientific references [13-16].

Five different concentrations (10, 25, 50, 100, and 200) mg/mL of each compound (Vitamin B1, 5-Sulphasalicylic acid, or EDTA-disodium salt) in sterilized water or (chloroxylenol, EDTA, or pentachlorophenol) in DMSO affording a clear solution were prepared.

Mueller- Hinton medium (containing 30% beef infusion, 1.75% casein hydrolysate, 1.7% agar, and 0.15% starch in (w/v) and adjusting to pH 7 at 25^{o}C) was used for all tested methods.25 mL of incubated test strain broth at 37^{o}C was activated in an incubator after 24 hrs. Pour – plate technique was applied for 0.2 mL of strain inoculation at (40-45) ^{o}C to solidify in Petri plates.

Filter paper disc method:

Each sterile paper disc was impregnated into a specific solution with known concentration, placed on the surface of the inoculated positive gram (*Staphylococcus aureus*) or negative gram (*Escherichia coli* or *Pseudomonas aeruginosa)* medium. After incubation (24 hrs. at 35^{o}C), the inhibition zone was checked out. Also, sterile water or DMSO was tested with this method to estimate its antibacterial activity.

Holes or wells diffusion method:

Holes were made by cup-borer that inoculated with the tested solution. 10µL of each used solvent was tested to ensure its antibacterial inhibition zone as zero then the tested solutions were studied against the particular tested bacteria (*Staphylococcus aureus, Escherichia coli,* or *Pseudomonas aeruginosa*).

Spotting diffusion method:

A spot of 10 µL of the tested solution or solvent on Petri dish was placed on the agar seeded with bacterial inoculums (*Staphylococcus aureus, Escherichia coli,* or *Pseudomonas aeruginosa*) under inspection, allowed to be absorbed, and incubated for 24 hrs.

Our novel method (Direct Solid Disc):

Each tested compound was weighted, compressed with 15 tons manual hydraulic press, that used with FTIR analysis, to form a disc with 1.3cm in diameter, and placed on the bacterial inoculum medium. If the obtained disc fractured, a few milligrams(2mg) of high purity potassium bromide were added then the obtained mixture compressed.

The inhibition zone (in mm) was measured for each applied method after allowing the Petri dishes to be settled down to affirm the diffusion in the medium.

RESULTS AND DISCUSSION

In many national and international published scientific works, hole or paper disc diffusion and dilution methods were applied to evaluate the antibacterial activity of newly prepared compounds with different functional groups[12, 17-23].

According to our observation, the main problem that faced many researchers here in Iraq and other countries is how to get a clear solution containing the target compound to qualify its antibacterial activity against particular human pathogenic bacteria[24-27]. Many derivatives were cancelled from antibacterial assessments for this reason.

A Spanish scientific group pressed of 0.05g hydroxyapatite and nanosilver-hydroxyapetite composite with 100 MPa to form 8 mm in diameter pellets and located each pellets at the centre of Petri plate to investigate their action against Gram-positive and Gram-negative bacteria. Their results ranged from(17-18) mm and the researchers considered their materials as strongly active antibacterial and biomaterial against *Staphylococcus aureus*, *Pneumococcus*, and *Escherichia coli* and can be used in implant and reconstructive surgery applications. The choice of this method due to the suspension formation of the tested materials[28].

In this work, several primary points have been taken in our consideration and setup as below:

1. The selection of compounds was mainly depended on their known antibacterial activity (not newly prepared) and these compounds were supplied from familiar trusted companies with high purity.
2. Some of the selected compounds were soluble in water while the others were insoluble (soluble in DMSO) to verify the solubility in water on inspected bacteria.
3. The ability of these compounds to form a disc without smashing was checked. Also, the need

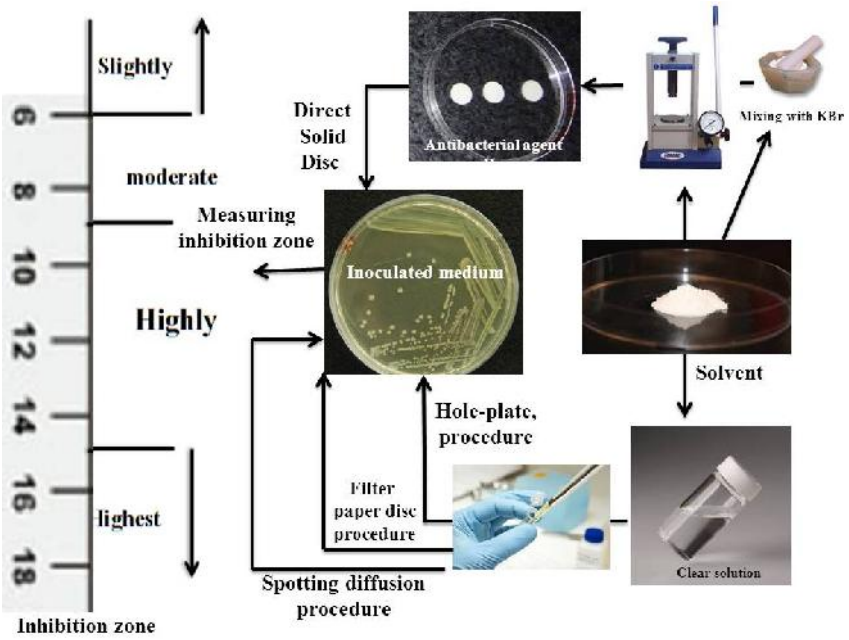
to mix potassium bromide with the fractured disc was tested.

4. For the above point (3), an initial antibacterial testing of potassium bromide disc against all selected bacteria was done and gave a encourage result (no inhibition zone).
5. The choice of potassium bromide depends on a simple fact that any added materials to a specific antibacterial agent have to be non- inhibited zone against the particular bacterium species and not nutritive to the tested bacteria.
6. Several elementary antibacterial tests with VitaminB1 (Soluble in water) with and without potassium bromide against all tested bacteria were performed and showed no significant changes in inhibition zone.
7. Also, Vitamin B1 with filter paper disc, well agar, and spotting diffusion methods was a preliminary selection to appoint the nearest method to our newly approached method (Direct Solid Disc). In this point, our opinion gathered that spotting diffusion method is the nearest method to our approach.
8. For water soluble compound, first direct solid disc experiments showed that using one Petri dish for more than one disc gave confused observation as a result of inhibition overlapping.

Scheme -1- summarizes the differences between the standard antibacterial and our approached procedures. It shows that our newly procedure eliminated many workable steps starting from choosing non-toxic solvent for preparing clear solution (with important character of permitting bacterial growth) to other feasible steps of hole-plate, filter paper disc, or spotting diffusion method.

Table -1- shows the inhibition zone of three compounds (chloroxylenol, EDTA, or pentachlorophenol) soluble in DMSO by applying spotting diffusion method. Also, Table -2- shows the inhibition zone of three compounds (Vitamin B1, 5-Sulphasalicylic acid, or EDTA-disodium salt) soluble in sterile water by applying spotting diffusion method.

By applying the new direct solid disc approach, more significant results were observed as shown in tables -3- and -4-. From the tables (1-4), it can be noticed that this novel applied method (Direct Solid Disc) was more detectable of the tested material with accuracy and precision in antibacterial screening subject than the spotting method results. Also, it can be concluded that this (Direct Solid Disc) gave a direct visual illustrative image of any tested material with its known weight as applied in this method and shown in figures (1 and 2).



Scheme -1 Direct Solid Disc and standard antibacterial testing procedures.

Table 1
Spotting diffusion results of chloroxylenol, EDTA, or pentachlorophenol soluble in DMSO.

Compound symbol	Concentration, mg/mL	Inhibition zone, mm		
		Staphylococcus aureus	*Escherichia coli*	*Pseudomonas aeruginosa*
A	200	35	20	34
	100	31	25	32
	50	28	19	30
	25	16	14	16
	10	15	14	17
B	200	35	40	16
	100	32	35	33
	50	31	30	30
	25	25	25	35
	10	20	20	20
C	200	40	32	45
	100	37	20	40
	50	40	27	36
	25	40	28	43
	10	40	26	40

A: Ethylene diaminetetraacetic acid (EDTA); B: Chloroxylenol; C: Pentachlorophenol

Table 2
Spotting diffusion results of Vitamin B1, 5-Sulphasalicylic acid, or EDTA-disodium salt soluble in sterile water.

Compound symbol	Concentration, mg/mL	Inhibition zone, mm		
		Staphylococcus aureus	*Escherichia coli*	*Pseudomonas aeruginosa*
D	200	20	*	15
	100	17	*	*
	50	*	*	*
	25	*	*	*
	10	*	*	*
E	200	30	28	33
	100	25	22	28
	50	22	22	25
	25	15	16	19
	10	*	*	*
F	200	21	13	*
	100	17	15	*
	50	14	13	*
	25	17	11	*
	10	13	*	*

D: Vitamin B1; E: EDTA-disodium salt; F: 5-Sulphasalicylic acid * no inhibition was observed.

Table 3
Direct Solid Disc results of chloroxylenol, EDTA, or pentachlorophenol soluble in DMSO.

Compound symbol	Weight, mg	Inhibition zone, mm		
		Staphylococcus aureus	*Escherichia coli*	*Pseudomonas aeruginosa*
A	200	29	20	70
	100	30	27	60
	50	30	29	22
	25	40	32	60
	10	40	27	55
B	200	35	30	19
	100	35	25	15
	50	31	25	18
	25	32	23	20
	10	30	25	18
C	200	37	27	62
	100	30	45	23
	50	35	30	20
	25	40	25	20
	10	40	24	16

A: Ethylene diaminetetraacetic acid (EDTA); B: Chloroxylenol; C: Pentachlorophenol

Table 4
Direct Solid Disc results of Vitamin B1, 5-Sulphasalicylic acid, or EDTA-disodium salt soluble in sterile water.

Compound symbol	Weight, mg	Inhibition zone, mm		
		Staphylococcus aureus	*Escherichia coli*	*Pseudomonas aeruginosa*
D	200	62	35	32
	100	56	35	30
	50	50	30	30
	25	55	*	20
	10	45	*	18
E	200	65	85	65
	100	40	85	63
	50	75	85	58
	25	55	85	54
	10	42	55	42
F	200	78	70	65
	100	85	85	62
	50	85	85	55
	25	**85**	**85**	33
	10	**85**	**85**	32

D: Vitamin B1; E: EDTA-disodium salt; F: 5-Sulphasalicylic acid * no inhibition was observed.

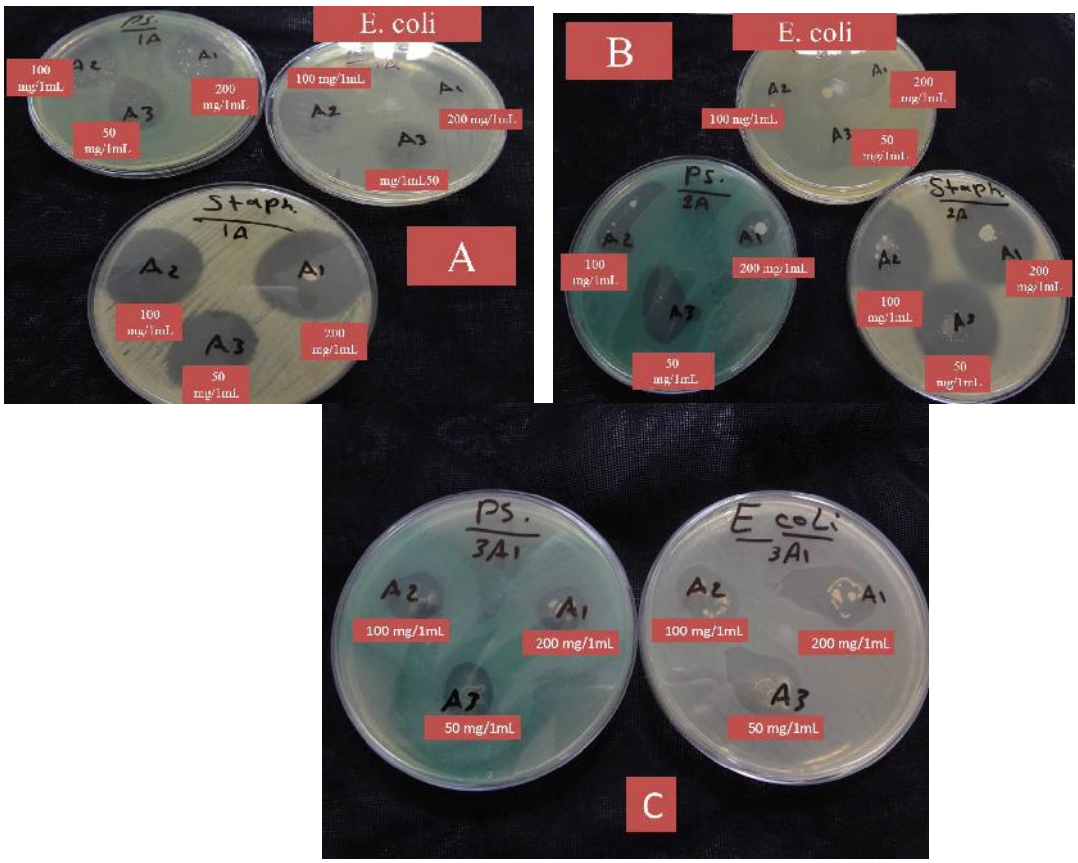


Figure 1
Antibacterial activity of the tested samples in DMSO at different concentrations with spotting method

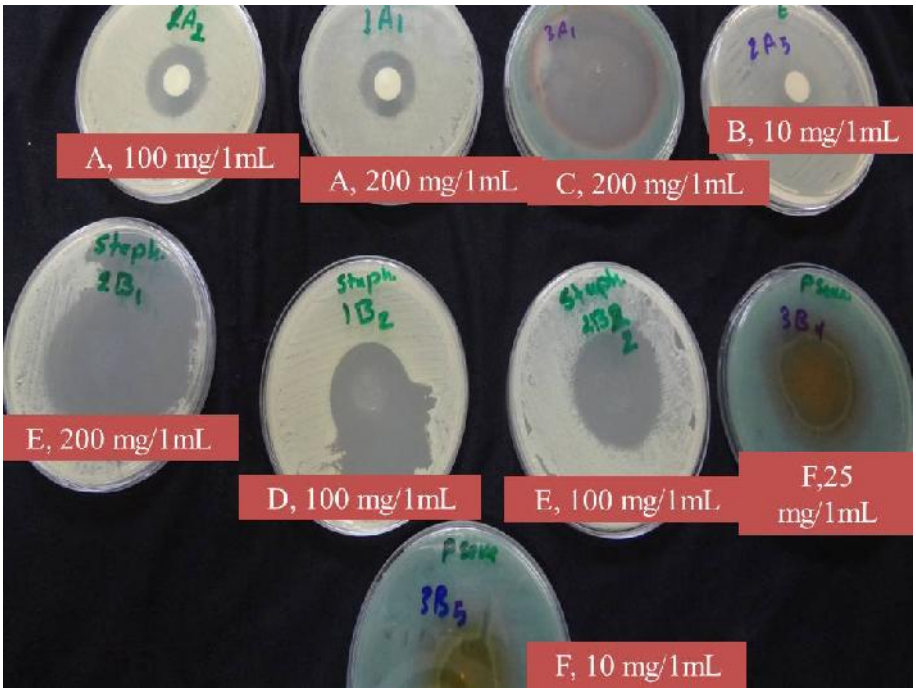


Figure 2
Antibacterial activity of the tested samples at different concentrations with Direct Solid Disc method.

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