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Effect of various solvents on bacterial growth in context of determining MIC of various antimicrobials

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Abstract

Dimethyl sulfoxide (DMSO) and ethanol are frequently used as solvent for natural as well as synthetic antibacterial compounds, in order to determine their MICs. Effect of these solvents on bacterial growth is an important factor to be considered, while considering reproducibility of experiments for MIC determination. Present study aimed at determining the effect of different concentrations (1% to 6%) of DMSO, ethanol, and methanol on the growth of five different bacteria. DMSO scored better followed by methanol and ethanol, in terms of their compatibility with MIC determination. Lower concentrations of solvents, which apparently do not affect the bacterial growth significantly, may still potentiate the effect of antibacterial compound under test.

Introduction

Due to rising incidence of drug resistance among pathogenic bacteria, new antibacterial compounds are constantly being searched5. Potency of any newly reported antibacterial preparation can be quantified and compared with those already known by determining its MIC value. Minimum Inhibitory Concentration (MIC) is defined as the lowest concentration of antimicrobial agent required to inhibit growth of a test organism over a defined interval related to the organism's growth rate, most commonly 18 to 24 h. MIC has been accepted as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism7.

Conventionally broth dilution method is applied for measuring the MIC. It requires preparing various dilutions of the compound under test in a suitable solvent. In case of natural products, generally extraction is carried out using solvents of varying polarity, ethanol and methanol are most commonly applied. To quantify antimicrobial activities, extracts have to be dried. Frequently it is difficult to resolubilize extracts even in the solvent originally used. In serial dilution assay the solvent has to be miscible with water. Water frequently doesn't dissolve the intermediate polarity or non-polar components of a dried extract. An alternative is to use solvents such as methanol, ethanol or DMSO. Selection of appropriate solvent is one of the most significant factors which can influence MIC measurements *in vitro*. Ethanol3 and DMSO6 are preferred since they are miscible with water. DMSO is a highly polar, stable substance with exceptional solvent property6. However, DMSO1, ethanol4 and other solvents used in various bioassays have been reported for their antimicrobial effect2. Thus it becomes essential to ensure that the final concentration of the organic solvent is not likely to interfere with the bioassay (MIC determination). It should also be noted that each organism may exert varying susceptibility to these solvents.

Present study aimed at determining the effect of different concentrations (1% to 6%) of DMSO, ethanol, and methanol on the growth of five common bacterial pathogens.

Materials and Methods

Microorganisms

Staphylococcus epidermidis MTCC 435, *Pseudomonas oleovorans* MTCC 617, *Vibrio cholerae* MTCC 3906, *Shigella flexneri* MTCC 1457 and *Salmonella paratyphi A* were used as test organisms. All the bacterial strains except *Salmonella paratyphi A* were obtained from the Microbial Type Culture Collection (MTCC), Chandigarh, India. *S. paratyphi A* was obtained from the Microbiology Dept., Gujarat University, Ahmedabad.

Growth Medium

Mueller Hinton (MH) Broth (HiMedia, Mumbai, India)

Solvents

Dimethyl sulfoxide (DMSO) (sd fine chemicals Ltd., Mumbai, India), methanol (Merck, Mumbai, India), and ethanol (Baroda chemical industries Ltd, Vadodara, India).

Procedure

Varying volumes of MH broth and respective solvent were mixed in different test tubes (table 1) so that concentration of the solvent ranged from 1-6 % v/v. To this was added 500 µL of inoculum, which was prepared from overnight growth of the test organism, so as to match the turbidity of 0.5 McFarland standard. Total volume in each of the tube was thus made 2 mL. A tube containing growth medium (1500 µL) was inoculated with 500 µL of inoculum, and put as growth control. Tube containing gentamicin (250 ug/mL; HiMedia, Mumbai, India) was used as positive control. An uninoculated tube of MH broth (2 mL) was put as sterlity control. Incubation was made at 35°C for 16-20 h, before optical density being measured at 625 nm (ELICO SL160 double beam UV-Vis spectrophotometer). Growth in each of the 'test' tube was expressed relative to that of 'control'.

Concentration of solvent	Volume added	
	Broth (µL)	Solvent (DMSO/Methanol/Ethanol) (µL)
1%	1480	20
2%	1460	40
3%	1440	60
4%	1420	80
5%	1400	100
6%	1380	120

Table 1: Volume of medium and solvent for each concentration

Results and Discussion

As evident from table 2, all the bacteria except *S. flexneri* exert little or no susceptibility to DMSO upto 2% concentration. *V. cholerae* and *P. oleovorans* remain unaffected even upto 3% DMSO. All bacteria tested exhibit significant decrease in

growth when exposed to DMSO at a concentration of 4% and beyond. *S. flexneri* seems to be more tolerant to higher concentrations of DMSO as ompared to other test organisms (fig.1).

Organisms	DMSO					
	1%	2%	3%	4%	5%	6%
	Growth (%) compared to control					
S. paratyphi A	100	100	93	50	33	12
S. epidermidis	98	97	85	37	21	15
S. flexneri	90	88	88	83	73	63
V. cholerae	100	100	100	49	46	43
P. oleovorans	100	100	100	42	35	33

Table 2: Effect of DMSO on bacterial growth

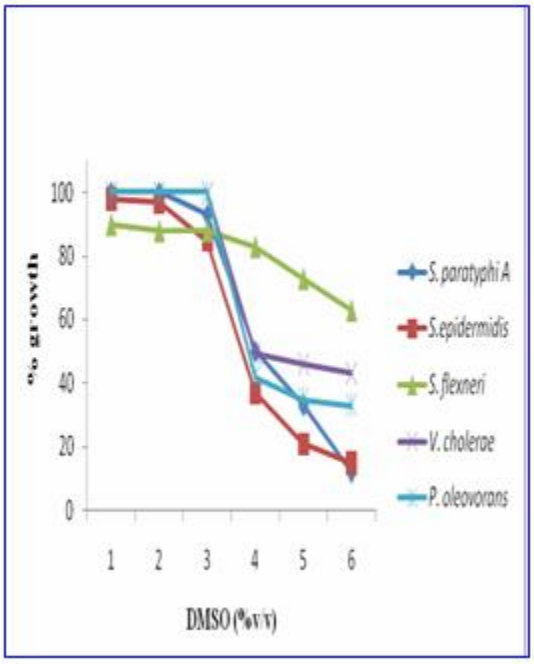

Fig. 1: Effect of DMSO on bacterial growth

As indicated in table 3, 1% methanol has little or no effect on bacterial growth except in case of *S. paratyphi A* and *S. flexneri*. *S. epidermidis* is the only bacterium which shows no significant susceptibility upto 3% v/v methanol. All bacteria (especially *S. paratyphi A*) tested exhibit significant decrease in growth when exposed to methanol at a concentration of 4% and beyond (fig.2).

Organism	Methanol					
	1%	2%	3%	4%	5%	6%
	Growth (%) compared to control					
S. paratyphi A	88	86	82	17	15	10
S. epidermidis	100	100	97	43	32	26
S. flexineri	92	91	90	83	70	58
V. cholerae	98	92	90	63	61	59
P. oleovorans	100	100	86	83	79	33

Table 3: Effect of Methanol on bacterial growth

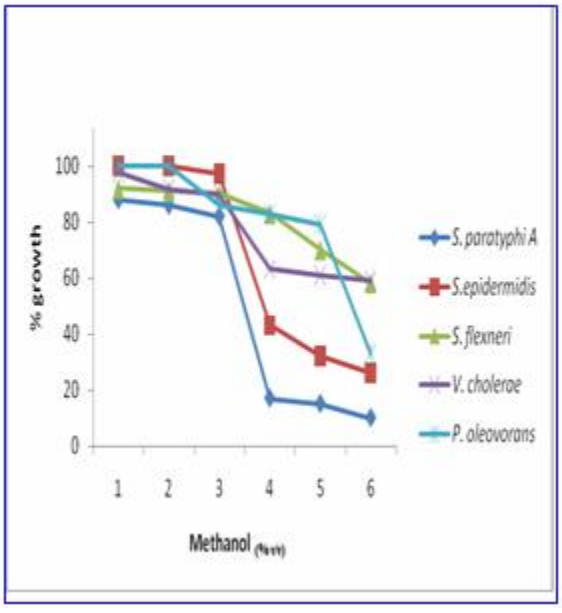

Fig. 2 Effect of methanol on bacterial growth

As reported in table 4, *S. flexineri* is the only organism whose growth remained unaffected by ethanol upto a concentration of 3%. Growth of all other bacteria was affected even at 1% level. *S. paratyphi A* exhibited the highest susceptibility towards ethanol at all concentrations (fig. 3).

Organism	Ethanol					
	1%	2%	3%	4%	5%	6%
	Growth (%) compared to control					
S. paratyphi A	59	41	37	24	10	4
S. epidermidis	91	80	70	54	34	26
S. flexineri	100	100	100	72	61	36
V. cholerae	79	78	75	67	46	43
P. oleovorans	76	72	59	57	55	44

Table 4: Effect of Ethanol on bacterial growth

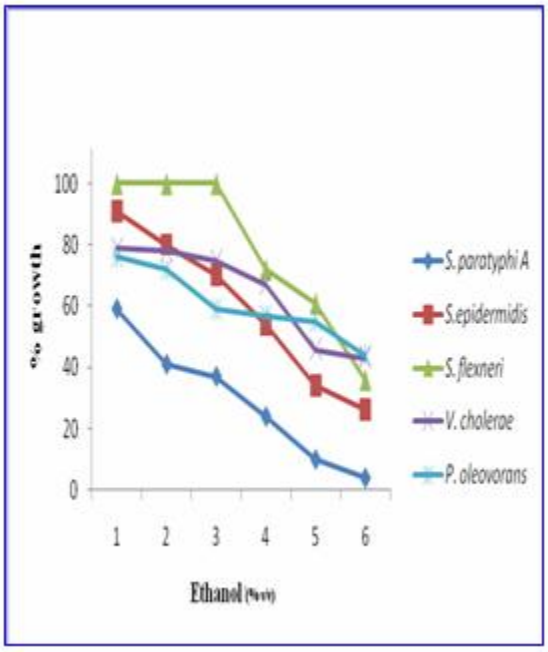

Fig. 3: Effect of Ethanol on bacterial growth

When average values of % growth of all organisms are compared at a given concentration of all the three solvents (table 5), it becomes clear that DMSO scores better over methanol and ethanol. Ethanol seems to be the poorest as its inhibition potential is higher than that of DMSO and methanol, at all concentrations tested. However DMSO and methanol also exert high inhibition potential at concentrations of 4% and beyond. Interestingly DMSO is less toxic at 1-3 % than methanol, but it is the other way in the concentration range of 4-6 %. On an average, at 5% level, both DMSO and ethanol exert almost identical toxicity. Though DMSO and ethanol are generally considered safe below 3% v/v3, present study suggests that it can not be accepted as a general fact for all test organisms..

Solvent	**Average % growth for all organisms**					
	1 %	2%	3%	4%	5%	6%
DMSO	97.6	97	93.2	52.2	41.6	33.2
Methanol	95.6	93.8	89	57.8	51.4	37.2
Ethanol	81	74.2	68.2	54.8	41.2	30.6

Table 5: Inhibition potential of different solvents at varying concentrations

Conclusively, if a biological test is to be performed, care should be taken that the solvent giving best solubility is compatible with the system. Non-aqueous solvents may prove toxic for the test organisms. Tests to determine the concentration of solvent above which toxicity occurs should always be carried out before the experiment proper, and controls with potential solvent toxicity in mind should be incorporated into the experiment. It is of utmost importance to ensure that the final concentration of the organic solvents is not likely to interfere with the bioassay. Further, solvent suitable with one test organism may not be proper for use with another, because different organisms respond differently to same concentration of a given solvent, as apparent in the results present above. It is advisable to keep the concentration of any organic solvent at the lowest possible level in the assay system, however it may prove difficult in case of few natural products, when initial extraction efficiency is poor. Even the lower concentrations of these solvents which have no apparent effect on bacterial growth, may still potentiate the effect of antibacterial compound under test.

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