

## **Evaluation of Antibacterial Activity of Disulfiram.**

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**Abstract:** Antibacterial activities of several ‘non-antibiotic drugs’ used in treatment of a variety of non-infectious human diseases have been observed. Such an effect has been noticed for barbiturates, beta-adrenergic receptor antagonists, diuretic drugs, H<sub>1</sub> antihistamines, mucolytic agents, nonsteroid anti-inflammatory drugs, proton pump inhibitors and psychotherapeutic drugs. A synergic or antagonistic effect with antibiotics of several of these drugs have also been noticed. In this overview the knowledge in the field is summarized, omitting hormones, drugs with local effects and those with low therapeutic indexes. Therapeutic implications and future considerations are discussed. Anti alcoholism drug Disulfiram having diverse pharmacological action, were screened for possible antibacterial property against known seven sensitive bacteria, belonging to Gram positive and Gram negative types. Disulfiram were seen to possess pronounced antimicrobial property. The minimum inhibitory concentration (MIC) of disulfiram [1,1',1",1"-[disulfanediylbis (carbonothioylnitrilo) tetraethane] was determined by disc diffusion method, which ranged From 1-2 Mg/Ml In Most Of The Strains, While Some Strains Were Inhibited At Even Lower Concentration.

**Keywords:** Disulfiram, Antibacterial activity, nutrient agar medium, disc diffusion method.

### **Introduction:**

Many microorganisms produce infections in humans and other organisms. These infections are at present treated by using antibiotics and other antimicrobial agents. However, many microorganisms develop “resistance” to the above compounds due to their indiscriminate use. So, it is essential to develop a new antibiotic/antimicrobial compounds to combat the menace of resistance developed by microorganisms. But developing a new antibiotic or a antimicrobial compound and bringing them to market involves of lot of time, labour, money and failures. So, researchers across the world are trying to discover the antimicrobial activities of some drugs which are used for treating other diseases or disorders. Some success is achieved in this regard. This article discusses the antimicrobial activities of some anti hypertensives, tranquilizers, analgesics, local anaesthetics and their possible role in treating infections. In pharmaceutical microbiology is having major role to identify the newer anti biotics from existing non anti biotic categories. Now a days very large number of anti biotics and anti bacterial chemotherapeutics exist today, their usage is becoming restricted not only because many of them produce toxic reactions but also due to emergence of drug resistant bacteria. The drug disulfiram[1,1',1",1"-[disulfanediylbis(carbonothioylnitrilo)tetraethane] use as a antialcoholism and the recent study shows the anti fungal and anti tubercular activity.[1,2]

### **Materials And Methods**

#### **Chemicals and Reagents:**

Disulfiram was obtained as pure dry powder form from Lot No.: 10696 [50G] MFG Date: 2011 Farchemia Srl, Italy. Nutrient agar was obtained from Hi Media Pvt Ltd, Mumbai, India. Solvent methanol and

diethyl ethyl ether obtained from SD Fine Chem Limited, Mumbai. All glasswares were procured from M/S Borosil Ltd., Mumbai.

### **Collection of microorganism:**

Staphylococcus aureus ATCC No.29737, Bacillus subtilis ATCC NO. 6633, Escherichia coli ATCC NO. 8739, Micrococcus luteus/flavus ATCC NO. 10240, Pseudomonas aeruginosa ATCC NO.25619, Klebsiella pneumoniae ATCC NO. 10031, Bacillus pumilus ATCC NO. 12228 were obtained from National chemical laboratory, Pune, India.

### ***In vitro screening of Disulfiram for detection of antibacterial activity:***

Gram positive bacteria and Gram negative bacteria were sub cultured in nutrient agar slants. The tubes were incubated at 37°C, examined for appearance of growth after 24 h and stored aseptically. The drug was dissolved in sterile distilled water to get concentrations of 5,10,15, 20, and 25mg/ml, under aseptic conditions[3]. From the above sub-cultures, the organisms were directly suspended in 5mL of sterile distilled water. The turbidity of each suspension was adjusted to 0.5 McFarland Standard[4,5]. The suspensions were further diluted 1:100 with sterile distilled water. The inocula were mixed with molten nutrient agar medium(40°C) and poured into sterile Petri dishes(97mm in dia) aseptically and allowed to solidify. The drug dilutions(5...25mg/ml) were impregnated into sterile filter paper disc and kept on the surface of the media and incubated at 37°C for 24 hour and then the diameters of zones of inhibition were measured[6]

### **Determination of the minimum inhibitory concentration (MIC) of Disulfiram.**

Minimum Inhibitory Concentration (MIC) of Disulfiram was determined by employing concentrations 1-5mg/ml of the drug. The procedure which is mentioned above was adopted to obtain zones of inhibition[3-6] The results are presented as Table-2 and as figures 1-7

### **Result and Discussion:**

**Table-1 Results of *in-vitro* antibacterial activity of disulfiram.**

<b>Name of the organism.</b>	<b>Zone of inhibition(mm) Average±S.D*</b>				
	<b>5mg/ml</b>	<b>10mg/ml</b>	<b>15mg/ml</b>	<b>20mg/ml</b>	<b>25mg/ml</b>
Staphylococcus aureus	8±0.752	8±0.814	11±0.732	11±0.752	14±0.752
Bacillus subtilis	8±0.752	8±0.752	9±0.732	9±0.752	10±0.752
Micrococcus luteus/flavus	10±0.752	12±0.752	12±0.836	14±0.547	14±0.632
Bacillus pumilus	12±0.814	13±0.513	13±0.752	14±0.836	14±0.894
Escherichia coli	11±0.752	13±0.894	13±0.752	13±0.836	14±0.752
Pseudomonas aeruginosa	10±0.516	11±0.894	11±0.516	12±0.516	14±0.547
Klebsiella pneumoniae	9±0.816	10±0.516	13±0.632	13±0.516	13±0.408

\*n=6 p=0.05

**Table-2 Results of determination of MIC of disulfiram.**

Name of the organism.	Zone of inhibition(mm) Average±S.D*				
	1mg/ml	2mg/ml	3mg/ml	4mg/ml	5mg/ml
<i>Staphylococcus aureus</i>	6±0.732	7±0.836	7±0.732	8±0.752	9±0.752
<i>Bacillus subtilis</i>	6±0.752	7±0.752	7±0.732	8±0.752	8±0.752
<i>Micrococcus luteus/flavus</i>	6±0.752	7±0.752	8±0.836	9±0.547	10±0.632
<i>Bacillus pumilus</i>	6±0.814	6±0.513	7±0.752	8±0.836	9±0.894
<i>Escherichia coli</i>	6±0.752	7±0.894	7±0.752	8±0.836	8±0.752
<i>Pseudomonas aeruginosa</i>	6±0.516	7±0.894	7±0.516	8±0.516	9±0.547
<i>Klebsiella pneumoniae</i>	6±0.816	6±0.516	8±0.632	8±0.516	10±0.408

\*n=6 p=0.05.

## Discussion

From the above results, it can be observed that disulfiram (antialcholic agent) exhibited consistent antibacterial activity against strains of *Staphylococcus aureus* ATCC No.29737,*Bacillus subtilis* ATCCNO.6633, *Micrococcus luteus/flavus* ATCCNO. 10240, *Bacillus pumilus* ATCCNO.12228(all gram positive bacteria), *Escherichia coli* ATCCNO 8739, *Pseudomonas aeruginosa* ATCC NO25619, *Klebsiella pneumoniae* ATCC NO 10031(all gram negative bacteria)[7] Result shows that amitriptyline inhibits the growth of bacteria (both gram positive and gram negative) by disc diffusion method . The antibacterial activity is more pronounced in cases of gram positive bacteria than gram negative bacteria. The reason may be the better accessibility of cell walls of gram positive bacteria than that of gram negative bacteria, by disulfiram. The MIC of the drug ranges from 1-2mg/ml, which indicates the suitability of the drug for treatment of infections[8,9]

The drug disulfiram inhibits bacterial growth at 37°C had the most pronounced antibacterial effects . This drug is in routine therapeutic usage satisfying human toxicity tests, in course of time, be developed as the second or even the first line antimicrobial agent in many infections;(which is eflected from its MIC values) such properties would further enhance its applicability in humans[10]. Thus, the present study suggests that disulfiram has a potential for being developed into a powerful antimicrobial agent, the efficacy of which may be enhanced further by various structural modifications and clinical or chemotherapeutic synergistic combinations of the drug with conventional antimicrobics and/or non-antibiotics.[11,12]

**Fig 1: Antibacterial activity of Disulfiram against *Klebsiella pneumonia***

**Fig 2: Antibacterial activity of Disulfiram against *Escherichia coli***



**Fig 3: Antibacterial activity of Disulfiram against *Bacillus pumilus***



**Fig 4: Antibacterial activity of Disulfiram against *Staphylococcus aureus***



**Fig 5: Antibacterial activity of Disulfiram against *Pseudomonas aeruginosa*;**



**Fig 6: Antibacterial activity of Disulfiram against *Bacillus subtilis***



**Fig 7: Antibacterial activity of Disulfiram against *Micrococcus luteus/flavus***



### Conclusion:

The problem of bacterial resistance to almost all antibiotics calls for the necessity of finding out newer drugs. Although, there are continuous reports on newer antimicrobial agents, the search in completely different approaches have indicated antimicrobial property in a large number of compounds[13]. Disulfiram is used as an adjunct in the treatment of chronic alcoholism. Since Disulfiram contains four sulphur atoms in its structure, the drug was screened for its anti-bacterial activity against the selected bacteria. Upon screening, the drug has shown better activity against the chosen Gram positive bacteria than against Gram negative bacteria. So, it can be concluded that Disulfiram possesses antibacterial activity and can be further tested against pathogenic strains for further therapeutic use as an antibacterial drug.[2,14,15]

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