Test of Zantac Mutagenicity on E. coli strain

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Abstract

Results of this study were summarized by finding Minimum inhibitory concentration (MIC) of 8-azaguanine and zantac on the locally isolated normal flora strain E. coli that was identified by vitek-2 and 16s RNA . MIC of 8-azaguanine was reached 500 µg / ml comparison with MIC of zantac which was 2250 µg / ml. The ratio of resistance to 8-azaguanine was increasing in a direct proportion to the increasing concentration of zantac that was added to media. The resistance of bacteria reached 82% at the concentration of 1500 µg / ml of zantac. Furthermore testing the mutagenicity of zantac by antibiotics was showed that zantac resistance wasn't affected by trimethoprim-sulfamethoxazole (SXT) and azithromycin. Whereas sensitivity of E.coli was decrease towards amikacin and gentamicin, (aminoglycosides antibiotics) was decreasing during the increase of zantac concentration this gives evidences the zantac mutagenicity on DNA or RNA molecule by increasing its concentration on E. coli in the current study.

Keywords: *8-Azaguanine, Antibiotics, Inhibition zone, Resistance, Zantac, Mutagen.*

Introduction

The living organisms have been exposing to various chemical compounds that are mainly made by humans. Metabolism of these chemicals within living bodies is an addition possible factor for being carcinogens (Kesari, 2019). Among these chemicals, pharmaceuticals are used to treat a specific disease or dysfunction in the body of an organism (Atanasov et al., 2015). Zantac is an anti-treatment from H2 receptor antagonist(Fedorowicz et al., 2012) it is a drug approved by the US Food (US-FDA) and Drug Administration for the treatment of many digestive disorders such as stomach ulcers, duodenal ulcers, and Zollinger- Ellison syndrome (Ameen et al., 2006; Brogden et al.,1982). However, recently a problem has arisen regarding to this drug whether it is mutagenic or not while most studies wonder about additional researches to confirm or deny its mutagenic potential (Iwagami et al., 2021).

Therefore, the current study aims to the test mutagenicity of zantac on the Escherichia coli by examining the resistance or sensitivity to the 8-azaguanin and antibiotics with or without zantac drug.

Materials and Methods:

Test organism:

Stool specimens were collected from Al-Salam hospital/Mosul city, isolation was occurred in biology Dept. Sciences College / Mosul University. Colonies of bacteria were sub cultured many times for confirmation from its purity. MaCconkey agar, blood agar, Eosin-Methylene-Blue agar (EMB), Gram stain and biochemical tests were used for

identification, VITEK2 and 16s RNA techniques also was used for the identification.

8-Azaguanin Experiments

8-Azaguanine with 98% purity was used in this study, which supplied from Shanghai pengteng fine chemical co., ltd. the Stock solution was prepared by dissolving (0.25gm) in the smallest amount as possible of 1N of NaOH then completed to 100 ml D.W to reach a final concentration 2500 µg/ml (Hoffman and malling, 1974). The test was performed by adding ascending concentrations of 8-Azaguanin to minimal medium (MM) which was prepared according to (Atlas, 2010). Growth was compared with the control culture in each concentration after 24h.

Antibiotics sensitivity test

Amikacin (AK) and gentamicin (GN) are used with concentration 10 µg/disc. Trimethoprim-sulfamethoxazole (SXT) 25µg/disc and azithromycin (AZM) 15µg/disc also are used. The disk diffusion method was used to test antimicrobial resistance for E. coli (Vandepitte et al., 2003), as recommended by the Clinical Laboratory standards Institute (CLSI, 2022).

Zantac Medication

Zantac tablets were used with 150 mg concentration for each tablet .It is supplied from Sun pharmaceutical industries ltd. The empirical formula for ranitidine hydrochloride is C13H22N4O3S.H2Cl, its molecular weight is 350.9, chemical design of zantac is NN-dimethyl-5- (2- (1-methylamine -2-nitrovinyl amino) ethylthiomethyl) furfury lamine. With commercially name is Histac (Katzung et al., 2012).

MICs of Zantac

They are measured by dissolving one tablet of zantac in 10 ml D.W to get 15000 µg /ml stock solution. Progressive concentrations from this stock solution were added to MM media. Cultures were done with overnight broth E. coli. Control media also are cultured for comparing bacterial growth with or without zantac.

Zantac mutagenicity analysis

By 8-Azaguanine presence:

This test was done by culturing overnight bacterial broth on MM media which contained final MICs concentration (370, 750, 1500, and 2250) µg/ml of zantac. Whatman filter No.1 and 5mm discs are saturated with killing concentration of 8-azaguanine. Inhibition zones were recorded and this experiment repeats for 3 times. Control media that didn't contain zantac but only discs of 8-Azaguanine also were done to compare growth.

By Antibiotics presence:

Mutagenicity of zantac was also measured by using antibiotics resistance method that E.coli previously was recorded as sensitive to it. Measurements are occurred in the same way as 8-azaguanine only the inhibition zones of antibiotics discs were calculated with or without zantac (control).

Result:

MICs of 8-Azaguanine and zantac

Table 1: Growth rates estimation of E. coli for one day on the MM medium with increased concentrations of 8-Azaguanine and zantac.

Concentration of µg/ml		The growth rate on MM		The growth rate on MM only
8-Azaguanine	**zantac**	**8-Azaguanine**	**zantac**	
32	50	+++	+++	+++
62	100	+++	+++	+++
125	250	+++	+++	+++
190	500	++	+++	+++
220	750	++	++	+++
300	1000	+	++	+++
400	1250	±	+	+++
500	1500	±	±	+++
	2000	±	±	+++
	2250	-	-	+++

Excellent growth +++, very good growth ++, little growth+, very little growth±, no growth-.

1. Mutagenicity of Zantac measured by 8-Azaguanine

Table 2: Inhibition diameters and percentages of resistance to ascending concentrations of Zantac inoculated by wt. E. coli with sub lethal of 8-Azaguanine discs.

Concentration of zantac µg/ml	**Concentration of 8-Azaguanin µg/ml**	Diameters of inhibition zones (mm)	**The percentage of Zantac resistance**
0	300	15	**12%**
500	300	12	**20%**
750	300	10	**25%**
1250	300	8	**42%**
1500	300	6	**82%**

2. Mutagenicity of Zantac measured by Antibiotics

Table 3: Inhibition zones measurements of E. coli against antibiotic discs with or without Zantac.

Antibiotics	Zantac Concentrate g/ ml µ	Inhibition zone(mm) Stage.2
Amikacin (AK)	0	**20**
	500	**18**
	750	**16**
	1500	**12**
	2250	**6**
Gentamicin (GN)	0	**23**
	1500	**20**
	750	**20**
	1500	**15**
	2250	**8**
Trimethoprim-Sulfamethoxazole (SXT)	0	**30**
	500	**30**
	750	**30**
	1500	**30**
	2250	**30**
Azithromycin AZM)(	0	**20**
	500	**20**
	750	**20**
	1500	**20**
	2250	**20**

Figure1: amplicons of 16s rRNA gene amplified using universal primers.

G1, 2 and 3 replicates of E.coli 16s rRNA genes.

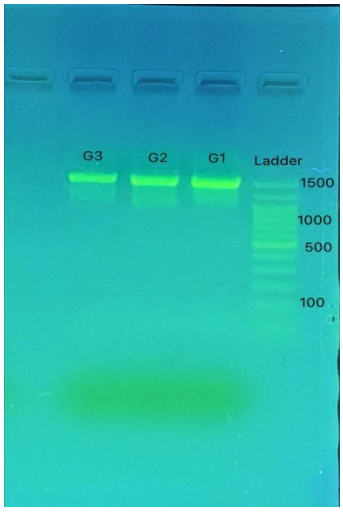


Figure 2: control media without zantac only antibiotics. A: gentamicin, B: amikacin

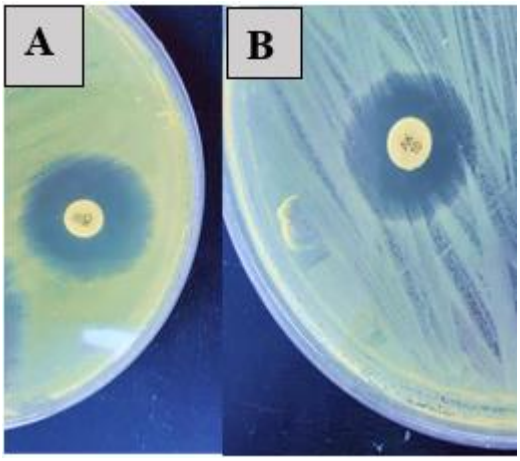
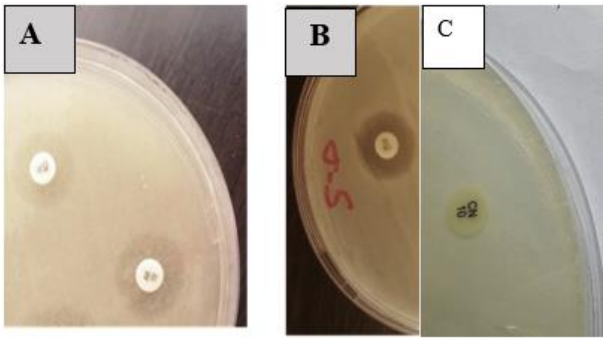


Figure 3: Decreased inhibition diameter with increased concentrations of zantac drug from A to B and finally C.



Discussion:

Molecular diagnosis by 16s RNA

After diagnosis by vitek-2 system, it is essential to confirm this identification by molecular level. The universal primers 27F and 1522R were used to amplify the 16s subunit. The size of gene was 1500bp as Fig 1.

MICs of 8-Azaguanin

A wide range of concentrations were tested to find the final lethal concentration according to (Table 1), in which there was a gradual growth decreasing until a complete killing of cells. This indirect killing indicates the inclusion of the compound within the composition of

nucleic acids by formation hydrogen bonds , which leads to the stable unit of 8-azaguanin within nucleic acids structure (He and Seela, 2003). The ability of 8-azaguanin to embed within nucleic acids is recently confirming through X-ray examinations and by specialized chemical reactions with chemical reagents that have high significance (Reu et al., 2019). The increase in inclusion led to the killing of E. coli cells in the end in our current research 500 µg/ml (Table 1). This concentration is approximately close to the least growth concentration occurred of the E. coli k-12 strain but it's growth measured by using the liquid medium method (Tsukamura and Tsukamura, 1963).

However, 8-Aza ultimately kills cells because it inhibits the protein biosynthesis by interfering with the mRNA synthesis; it prevents more than one interaction in the new peptide bonds (Albert, 1986; Zimmerman, 1968).

MIC of zantac:

When the zantac drug was added at a concentration 500 µg/ml this concentration did not affect the growth of bacteria (Table 1), until we reach to killing concentration 2250 µg/ml which kill the bacteria on MM media alone comparing with control media.

Zantac Mutagenicity measured by 8-Azaguanin

It is visible from (Table 3) that when we increase the concentration of zantac, the resistance of 8-aza increases, the percentage of zantac resistance was calculated from many repeated experiments. Furthermore, different concentrations were applied taken on E. coli bacteria but the control media were not contain zantac just 8-aza. The controls give sensitivity with 15 mm diameter and 12% inhibition percentage.

The resistance increases with increasing of resistance by 20% (Table 2), which consider a rather small percentage. At a concentration of 750 µg / ml from zantac, the resistance rate rise to 25% as well as with the concentration 1250 µg / ml which is reached to 42% (Table 2).

Finally, at the concentration 1500 µg / ml (less than the lethal concentration) the resistance reached to its higher level. The diameter of the inhibition in most colonies reached 6mm (Table 2), and sometimes it is completely adjacent to the saturated filter disc of 8-aza. From above, we conclude this experiment which based on Ames test principle gives result is the higher concentration of drug led to higher resistance in E. coli as a test organism. Another study found similar result that increasing in zantac concentration cause 43% damage in seminal tubes tissue also other variation noted at hormones levels (Oraibi, 2019).

The reason for mutagenicity of zantac it's containing oxygen and nitrogen ions. Sometimes is referred to it as N-nitrosodium dimethylamine which is a probable carcinogen molecule (Iwagami et al., 2021). When it interacts with stomach acidity, it will turn into toxic derivatives that lead to bases substitution. This state in E. coli known is errors frame and shift errors (Flora et al., 1983). Therefore, the current research proves the efficiency of the 8-aza system in testing mutagenicity of chemicals. This also proved in other research which showed the tested chemicals in the Ames system are not mutagenic while they appeared mutagens when tested with the 8-aza system (Bignami and Crebelli, 1979).

Although some research indicates that there are no potential risks from zantac when patients are followed for several years, but in the same time, they recommend more accurate studies on the patient who are regularly treat by zantac.

Zantac Mutagenicity measured by Antibiotics

It was observed from (Table 4) that the sensitivity to amikacin started to decrease with increase of zantac concentration and the same happened with gentamicin (Figs 2, 3) which is also considered as aminoglycoside. Both of them targets protein synthesis interfering with (rRNA) ribosomal ribonucleic acid (Krause et al., 2016).

The bacterial resistance to these antibiotics is a result of methylation and modification on 16s rRNA that lead to inhibition of aminoglycoside binding (Wachino and Arakawa, 2012). Also, resistant bacteria contain repetitive copies of rRNA[20]. Modification or methylation and presence of multiple copies of rRNA did not happen or it wasn't present in E.coli in the current study because it is sensitive to these antibiotics when zantac concentration was zero (Table 4).

From above, we conclude that zantac was able to make a mutation in the 16s rRNA molecule or prevent methylation and modification which lead to a reduction of E. coli susceptibility to aminoglycosides. Regarding trimethoprim, constancy of inhibitory diameter measurements was observed, despite the increase in zantac concentration (Table 4). Trimethoprim binds competitively with dihydrofolate reductase (Fol A) in the Folic acid pathway (Kwon et al., 2010).

Loss of zantac Mutagenicity in the presence of trimethoprim is because of multifaceted resistance of E. coli to this antibiotic. This resistance includes glycin production, SOS response, and protein production (Duployez et al., 2018). In this situation, zantac did not cause a mutation in this pathway and did not prevent the binding of trimethoprim to its target site which led to continuity of its active work against E. coli. The current strain E. coli has a high sensitivity to trimethoprim which was compatible with (Duployez et al., 2018), while the sensitivity is constant despite of the high concentrations of zantac.

E. coli also isn't able to resist azithromycin despite the increasing concentration of zantac and this may due to multifaceted resistance to azithromycin which includes: efflux pumps, multiple chromosomal mutations, and Macrolid-resistant genes that reach to 35 genes (Gomes et al., 2019).

Zantac may be able to influence on target site on rRNA (as with aminoglycosides) or target site on DNA and general RNA (as with 8-azaguanin). Both aminoglycosides and 8-azaguanin were used as evidences or reagents of the zantac effect. Zantac effects are also related to the increasing in drug concentration itself.

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