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The Curing Activity of 5-Fluorouracil (5-FU) and Its Role in Reducing The Antibiotic Sensitivity in Some Resistance Bacteria

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THE RESULTS showed that all the studied bacterial isolates had multiple resistance to the studied antibiotics before treatment with (5-FU). The current study aimed to investigate the curing activity of the anti-cancer drug (5-FU) and its effect on bacterial resistance to antibiotics. the results of the current study showed variation in plasmid content between different isolates (Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli). The results showed that all of the studied isolates lost their plasmids completely after treatment with sub-MIC concentration of the drug (5-FU). However, treatment with (5-FU) resulted in the loss of resistance of all isolates to the antibiotics to which they were resistant before treatment, the antibiotics (AM, ATM, FEP) became more affecting after treatment with the drug against *S. aureus* and (PRL, FEP, MEM, CIP) against *P. aruogenosa* compared with the rest of the antibiotics studied. It was concluded in the current study that the anti-cancer drug (5-FU) demonstrated a distinct plasmid curing ability and had a strong effect on the removal of bacterial resistance to antibiotics, which opens the way for the use of this drug in combination with antibiotics as therapeutic combination against the multidrug resistant pathogenic bacteria which may require conducting additional experiments to investigate the activity of this therapeutic combination.

Keywords: Curing, 5-Fluorouracil (5-FU), Antibiotics, Bacterial resistance.

Introduction

In recent years, due to the dramatic increase and global spread of bacterial resistance to several of commonly used antibacterial agents, studies have been directed to investigating new therapeutic alternatives that are effective against antibiotic-resistant bacteria [1]. Drugs other than antibiotics act in many different ways on the growth of microbes. They may have direct antimicrobial activity, increase the activity of antibiotics when co-administered, or alter the pathogenicity of microorganisms. In an era in which it is becoming increasingly difficult to find new antimicrobial drugs, it is important to understand these antimicrobial effects and their potential clinical implications [2].

Bacterial plasmids play an essential role in the transfer and spread of antibiotic resistance genes. Plasmids also contain genes that enhance the survival of these bacteria, and because they are small in size, they usually contain only a small number of genes with a specific function [3]. Antibiotic resistance plasmids and virulence plasmids pose a real threat to global health, so it is necessary to use different methods to remove plasmids, including the use of chemicals in specific concentrations [4].

Many drugs other than antibiotics, such as cancer drugs, can have antimicrobial properties, but their effect on bacteria in the context of infection and drug resistance has only recently begun to be explored [5]. 5-Fluorouracil (5-FU) is one of the most widely used antimetabolic chemotherapy agents in recent decades. It has been used as a first-line antineoplastic agent in the treatment of several types of cancer [6]. 5-FU exerts its anti-tumor action mainly by inhibiting the enzyme thymidylate synthase, which leads to

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the destruction of the pool of DNA nucleotides required for DNA replication inside cells. Another activity includes integration into RNA, which leads to the inactivation of its synthesis [7]. This study considers the first study used this drug to determine its role in plasmid curing and reducing the bacterial resistance. the current study aims to detect the curing activity of the anti-cancer drug (5-FU) and thus its effect on bacterial resistance to antibiotics.

Material and Methods

Bacterial isolates

Nine bacterial isolates were used in this study, 3 isolates for each type of studied bacteria (*P. aeruginosa, S. aureus, E. coli*). which previously isolated and diagnosed from Clinical sources in the Department of biology /College of Science/ University of Mosul, Iraq.

Antibiotics sensitivity test

The antibiotic discs were used in the current study supplied by (Bioanalyse/Turkey). The following antibiotics were used against *S. aureus* :Azithromycin (AZM) 15µg/disc, Gentamicin (CN)10µg/disc, Tobramycin (TOB)10µg/ disc, Levofloxacine (LEV) 5µg/disc, Cefixime (CFM)5µg/disc, Aztreonam (ATM) 30µg/ disc, Meropenem (MEM)10µg/disc, Imipenem (IPM) 10µg/disc, Ampicillin(AM) 25µg/disc, Cefepime (FEP) 10µg/disc, Ceftazidime(CAZ) 30µg/disc Ciprofloxacin(CIP) 10µg/disc. While the fallowing antibiotic were used against *P.aeruginosa* (Amickacin(AK) 10µg/disc, Tobramycin (TOB)10µg/disc, Aztreonam (ATM)30µg/disc, Cefepime(FEP) 10µg/disc, Ciprofloxacin(CIP) 10µg/disc Ceftazidime(CAZ) 30µg/disc, Meropenem (MEM)10µg/disc, Imipenem (IPM) 10µg/disc, Piperacillin(PRL)100µg/disc Ceftriaxone(CRO) 10µg/disc, Rifampin(RA) 5µg/disc, Levofloxacine (LEV) 5µg/disc.

The Disc diffusion modified Kirby-Bauer method on Mueller-Hinton agar medium was used in this study. The Clinical and Laboratory Standards Institute (CLSI) guidelines are used for interpretative the results [9].

Estimation of the plasmid DNA content

The plasmid content of the studied bacteria was estimated before and after treatment with the anti-cancer drug (5-FU) by using Promega PureYield™ Plasmid Miniprep System kit, the DNA plasmid was extracted and performing electrophoresis on an agarose gel at a concentration of 1.5% and a voltage difference of 80 volts for half an hour according to the method of [8].

Determination of the minimum ansubminimum inhibitory concentration of the drug (5-FU)

Th drug 5-Fluorouracil(5-FU)50mg/ml (Onko/ Turkey), was obtained from local pharmacies in Mosul city. The method [10] was adopted to prepare MIC and Sub-MIC by preparing serial dilutions (50, 25, 12.5, 6.25, 3.125 mg/ml) of the drug (5-FU) using distilled water. the (MIC) was determined as the lowest concentration of the inhibitory concentration at which the medium appears clear without turbidity. The (MIC) and (Sub-MIC) concentrations were confirmed by inoculating Mueller-Hinton solid medium with bacterial growth at these concentrations. The sub-MIC concentration showed clear growth while the MIC concentration did not show any bacterial growth.

Detection the effect of (5-FU) on antibiotics resistance and bacterial plasmid curing action:

The studied bacteria growing within the Sub-MIC concentration were re-tested for sensitivity to antibiotics and their plasmid content after treatment with the drug[8,9], to reveal the effect of the treatment on their resistance to antibiotics and their plasmid content.

Results

The results of the antibiotic sensitivity test showed that all the studied bacterial isolates showed multiple resistance to most the studied antibiotics before treatment with (5-FU).

The result of detecting the plasmid content of the bacterial isolates before treatment with the drug (5-FU) showed that *P. aeruginosa1* and *P. aeruginosa3* contain only one plasmid band, and *P. aeruginosa2* contain two plasmid bands, and also *S.aureus1* and *S. aureus3* contain one plasmid band, while *S.aureus2* and all *E.coli* isolates did not contain any plasmid band, as shown in **Table 1 and Figure 1**

P: *P.aeruginosa*, E: *E.coli*, S: *S.aureus* isolates.

TABLE 1. Plasmid content of the studied bacterial isolates

Isolates	NO. of plasmid bands
*P.*aeruginosa P1	1
*P.*aeruginosa P2	2
*P.*aeruginosa P3	1
*E.*coli E1	0
*E.*coli E2	0
*E.*coli E3	0
*S.*aureus S1	1
*S.*aureus S2	0
*S.*aureus S3	1

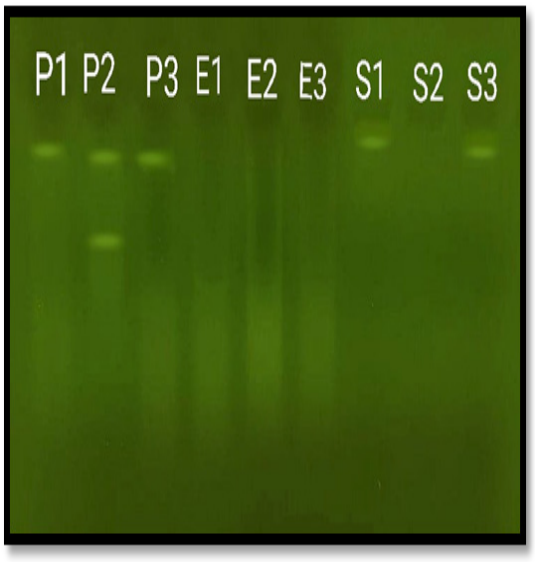

Fig. 1. Plasmid content of the studied bacterial isolates on agarose gel at a concentration of 1.5% and a voltage difference of 80 volts for half an hour.

The results of determine (MIC) and (SubMIC) of the anti-cancer drug (5-FU) against the studied isolates showed that the value of the MIC and SubMIC were the same for all isolates, with a concentration of 12.5 and 6.25 mg/ml, respectively.

The treatment with (5-FU) resulted in the loss of resistance of all isolates to the antibiotics to which they were resistant before treatment, the antibiotics (AM,ATM,FEP) became more affecting after treatment with the drug against *S.aureus* and the antibiotics (PRL, FEP, MEM, CIP) against *P.aruogenosa* compared with the rest of the antibiotics studied. The Table 2 showed the results of the antibiotic sensitivity test before and after treatment with the drug (5-FU).

Table 3 and Figure 2 showed the results of investigating the plasmid content of the studied isolates after treatment with the drug (5-FU). The results showed that all of the studied isolates lost their plasmids completely.

TABLE 2. Sensitivity of the studied isolates to antibiotics before and after treatment with anticancer drug (5-FU) (inhibition diameter in mm).

Isolates		Antibiotics											
		AM	FEB	AZM	CIP	MEM	ATM	TOB	CN	LEV	IPM	CAZ	CFM
S.aureus S1	B	(·)R	R(0)	R(13)	I(17)	S(29)	R (0)	S(20)	S(19)	I(16)	S(20)	R(9)	R(0)
	A	S(24)	S(27)	S(23)	S(37)	S(32)	S(28)	S(27)	S(26)	S(24)	S(28)	S(21)	S(19)
Isolates		**Antibiotics**											
		PRL	FEB	CRO	CIP	MEM	ATM	TOB	AK	LEV	IPM	CAZ	RA
P.aeruginosa P2	B	(·)R	(·)R	R(8)	R(8)	(·)R	R(14	(·)R	(·)R	(·)R	R(13	S(20)	(9)R
	A	S(32)	S(29)	S(28)	S(38)	S(35)	S(30)	S(25)	S(18)	S(27)	S(32)	S(37)	R(16)
P.aeruginosa P3	B	(·)R	(·)R	(·)R	(·)R	(·)R	(·)R	(·)R	(·)R	(·)R	(·)R	(·)R	(·)R
	A	S(28)	S(27)	S(30)	S(38)	S(36)	I (16)	S(23)	I(15)	S(30)	S(27)	S(22)	R(15)

TABLE 3. Plasmid content of the studied bacterial isolates after treatment with (5-FU).

Isolates	No. of plasmid bands	
	B	A
*P.*aeruginosa P2	2	0
P.aeruginosa P3	1	0
S.aureus S1	1	0

Before(B): After(A)

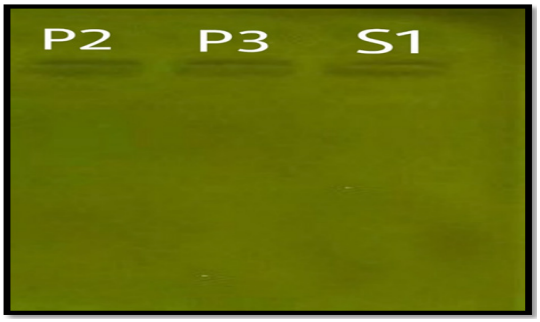

Fig. 2. Plasmid content of the studied bacterial isolates after treatment with (5-FU) on agarose gel at a concentration of 1.5% and a voltage difference of 80 volts for half an hour.

Discussion

The results of the current study showed variation in plasmid content between different isolates and sometimes between isolates of the same bacterial species , this variation in plasmid content may be due to the difference in both the source and geographical location of the isolate of the studied bacteria, as many factors play a role in the presence and transmission of plasmids in different bacterial species, as these plasmids are transmitted between different bacterial species and genera utilizing conjugation between bacterial cells [11]. Some multidrug-resistant bacteria contain plasmids of various molecular weights, while others do not contain plasmids, indicating that the latter's resistance was chromosomally carried [12]. This is consistent with the fact that some multidrug-resistant isolates had a single plasmid band during electrophoresis. While others did not contain plasmids, although all were multidrug resistant [13].

The results of determining the MIC and sub-MIC concentration of the drug (5-FU) showed that the values of these concentrations were similar for all the isolates studied, which reflects similar ability and mechanism of effect of this drug against these different isolates. The using of sub-MIC concentration of antibacterial agents aims to characterize the bacterial response that has been stimulated and allow cells to grow and survive [14].

Results of detection the plasmid content of the studied bacteria after treatment with the anti-cancer drug (5-FU) , showed that all of the studied isolates lost their plasmids completely, which reflects a very high activity in curing and removing plasmids. The loss of resistance of the studied bacterial isolates to antibiotics that were resistant to them before treatment with the drug (5-FU) revealed the extent of the effect of this drug on the various mechanisms through which bacteria can resist antibiotics, the most important of which is its effect on their plasmids [15].

It is clear from these results that antibiotic resistance genes may be carried on plasmids, and with the loss of plasmids, these bacteria lost their resistance to antibiotics. Several previous studies are consistent with our findings regarding loss of antibiotic resistance by loss of plasmids [16]. However, the phenomenon of plasmids curing may sometimes be insufficient to eliminate resistance, but it contributes to reduce the problem of bacterial resistance to antibiotics.

In our current study, the drug (5-FU) demonstrated a distinct ability to curing plasmids and had a strong effect on the resistance of Gram-positive and negative bacteria to antibiotics, which opens the way for the use of this drug in combination with antibiotics to remove resistance to these antibiotics. It is certain that the formation of such a therapeutic combination against the pathogenic bacteria that cause various infections requires conducting additional experiments, especially In-vivo, to investigate the activity of this therapeutic combination, according to our knowledge and research the current study consider the first study used this drug to determine its role in plasmid curing and reducing the bacterial resistance

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Conflict of Interest: None

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Author`s contribution: All researchers participated in designing the research. The first researcher carried out the practical aspect and statistical analysis. The second researcher completed the task of supervising, making tables, and writing.

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فعالية التحييد لـ -5 فلورويوراسيل (5-FU) ودوره في اختزال الحساسية الدوائية في بعض البكتيريا المقاومة

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أظهرت النتائج أن جميع العزلات البكتيرية المدروسة كان لديها مقاومة متعددة لأغلب المضادات الحيوية المدروسة قبل المعاملة بالدواء(5-FU).تهدف الدراسة الحالية إلى التحري عن فعالية التحييد للدواء المضاد للسرطان 5-Fluorouracil (5-FU) وتأثيره على المقاومة البكتيرية للمضادات الحيوية. أظهرت نتائج الدراسة الحالية تبايناً في المحتوى البلازميدي بين العزلات المختلفة (*Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli*) وفي بعض الأحيان بين عزلات نفس النوع البكتيري ، وأظهرت النتائج أن جميع العزلات المدروسة فقدت بلازميداتها تماماً بعد المعاملة بالتركيز المثبط تحت الأدنى sub-MIC للدواء (5-FU). كما ومع ذلك ، أدت المعاملة بالدواء (5-FU) إلى فقدان مقاومة جميع العزلات للمضادات الحيوية التي كانت مقاومة لها قبل المعاملة ، والمضادات الحيوية (FEP ، ATM ، AM) اصبحت أكثر تأثراً بعد المعاملة بالدواء ضد *S.aureus* والمضادات (CIP ، MEM ، FEP ، PRL) ضد *P.aruogenosa* مقارنة مع بقية المضادات الحيوية المدروسة.

نستنتج من الدراسة الحالية أن الدواء (5-FU)أظهر قدرة مميزة محيدة للبلازميد وكان له تأثير قوي على إزالة المقاومة البكتيرية للمضادات الحيوية ، مما يفتح الطريق لاستخدام هذا الدواء بالاشتراك مع المضادات الحيوية كتركيبة علاجية ضد البكتيريا المرضية المقاومة للأدوية المتعددة والتي قد تتطلب إجراء تجارب إضافية للتحقق من نشاط هذا المزيج العلاجي.

الكلمات الدالة: التحييد ، 5- Fluorouracil (5-FU)، المضادات الحيوية، مقاومة البكتيريا.