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Role of Some Antiemetics and Antispasmodics in the dissemination of antibiotic resistance genes among intestinal pathogenic E. coli

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Keywords:	Antibiotic resistance genes; non-antibiotics; anti-emetics; anti-spasmodic; conjugation; metoclopramide HCl; hyoscine butyl bromide; tiemonium methylsulfate.
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Competing Interests Use the instructions below to enter a competing interest statement for this submission. On behalf of all authors, disclose any competing interests that could be perceived to bias this work—acknowledging all financial support and any other relevant financial or non-financial competing interests. This statement is required for submission and **will appear in the published article** if	The authors have declared that no competing interests exist.

the submission is accepted. Please make sure it is accurate and that any funding sources listed in your Funding Information later in the submission form are also declared in your Financial Disclosure statement. View published research articles from *PLOS ONE* for specific examples. **NO authors have competing interests** Enter: *The authors have declared that no competing interests exist.* **Authors with competing interests** Enter competing interest details beginning with this statement: *I have read the journal's policy and the authors of this manuscript have the following competing interests: [insert competing interests here]* * typeset	
Ethics Statement Enter an ethics statement for this submission. This statement is required if the study involved: • Human participants • Human specimens or tissue • Vertebrate animals or cephalopods • Vertebrate embryos or tissues • Field research Write "N/A" if the submission does not require an ethics statement. General guidance is provided below. Consult the submission guidelines for detailed instructions. **Make sure that all information entered here is included in the Methods section of the manuscript.**	The study was conducted according to the guidelines of the Declaration of Helsinki, priori approval (No. 2/2022) by the ethical committee of Faculty of Pharmacy, Deraya University. Informed consent was not required since bacterial isolates were the subject of this study. Isolates, not human beings were studied. Thus, informed consent was not required by the local Ethics Committee

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Title Page

Role of Some Antiemetics and Antispasmodics in the dissemination of antibiotic resistance genes among intestinal pathogenic *E. coli*

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Role of Some Antiemetics and Antispasmodics in the dissemination of antibiotic resistance genes among intestinal pathogenic *E. coli*

Abstract

Background: Antibiotic resistance genes (ARGs) transfer rapidly among bacterial species all over the world contributing to the aggravation of antibiotic resistance crisis. Antibiotics at sub-inhibitory concentration induce horizontal gene transfer (HRT) between bacteria, especially through conjugation. The role of common non-antibiotic pharmaceuticals in the market in disseminating antibiotic resistance is not well studied.

Objectives: In this work, we indicated the effect of some commonly used non-antibiotics including antiemetic (metoclopramide HCl) and antispasmodics (hyoscine butyl bromide and tiemonium methyl sulfate) on the plasmid-mediated conjugal transfer of antibiotic resistance genes between pathogenic *E. coli* in the GIT.

Methods: Broth microdilution assay was used to test the antibacterial activity of the tested non-antibiotics. A conjugation mating system was applied in presence of the studied non-antibiotics to test their effect on conjugal transfer frequency. Plasmid extraction and PCR were performed to confirm the conjugation process. Transmission electron microscopy (TEM) was used for imaging the effect of non-antibiotics on bacterial cells.

Results: No antibacterial activity was reported for the used non-antibiotics. Plasmid-mediated conjugal transfer between isolates was induced by metoclopramide HCl but suppressed by hyoscine butyl bromide. Tiemonium methylsulfate slightly promoted conjugal transfer. Aggregation between cells and periplasmic bridges was clear in the case of metoclopramide HCl while in presence of hyoscine butyl bromide little affinity was observed.

Conclusion: This study indicates the contribution of non-antibiotic pharmaceuticals to the dissemination and evolution of antibiotic resistance in the environment at the community level. Metoclopramide HCl showed an important role in the spread of antibiotic resistance.

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Keywords: Antibiotic resistance genes, non-antibiotics, anti-emetics, anti-spasmodic, conjugation, metoclopramide HCl, hyoscine butyl bromide, tiemonium methylsulfate.

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1. Introduction

One of the most challenges or the biggest threats as described by the World health organization (WHO) is the emergence of antimicrobial resistance (AMR). As WHO said

that if no action is taken against the high increase in antibiotic resistance, more than 10 million people will die by 2050 [1]. Extensive use of antibiotics causes selective pressure on microbes giving rise to the emergence of resistant cells [2-7]. Horizontal gene transfer (HGT) is one of the major means of transferring or disseminating antibiotic resistance genes (ARGs) in the surrounding environment. Horizontal gene transfer can occur by conjugation, transduction, and transformation. Regarding conjugation, the exchange of genetic material between the donor and the recipient occurs by direct cell to- cell contact or via a connecting pilus [8, 9]. Typically, the exchange is mediated by mobile genetic elements, such as a conjugative plasmid. In addition, it was found that it is the most common way for disseminating antimicrobial resistance. Despite the spontaneous frequency of conjugation being rare, extensive use of antibiotics especially at concentrations lower than their MICs may lead to an increase in the frequency of the conjugation process [10, 11]. Accordingly, looking for unique conjugation inhibitors is an essential challenge in the fight against the spread of antibiotic resistance genes for improving the bacterial response toward antibiotics [12, 13]. Surprisingly, it was recently documented that the consumption of non-antibiotic prescribed drugs represents about ninety fifth of the drug market [14], the role of these prescribed drugs in the evolution of antibiotic resistance has received comparatively very little attention [15]. It remains unknown whether non-antibiotic prescribed drugs promote conjugation between bacterial strains or not which will be of clinical concern, as conjugative multidrug resistance plasmids enable quick expression of multidrug resistance phenotypes, therefore facilitating the emergence and widespread of antibiotic-resistant microorganisms [16]. Additionally, it's not clear whether there are common properties between the non-antibiotic prescribed drugs, or shared mechanisms that promote the ARGs horizontal transfer [11].

Gastro-intestinal tract (GIT) infection is one of the most common infections worldwide. *Escherichia coli*, a member of the microorganism family of Enterobacteriaceae, is the most prevailing commensal dweller of the gastrointestinal tracts of humans and warm-blooded animals, as well as one of the most important pathogens inflicting GIT infection [17]. Diarrheagenic pathotypes of *E. coli* (enterotoxigenic *E. coli* [ETEC], enteropathogenic *E. coli* [EPEC], enteroinvasive *E.*

Add with the reference 17 the following update reference after addition to GIT problem caused by H pylori and Clarithromycin resistance:-

Hussein RA, Al-Ouqaili MTS, Majeed YH. (2021). Detection of Helicobacter Pylori infection by invasive and non-invasive techniques in patients with gastrointestinal diseases from Iraq: A validation study. PLoS One. 23;16(8):e0256393. doi: 10.1371/journal.pone.0256393. PMID: 34424925; PMCID: PMC8382163.

coli [EIEC], and enteroaggregative *E. coli* [EAEC]) are highly detected [18]. Patients suffering from these infections receive antibiotics beside GIT concomitant pharmaceuticals such as, antiemetic and antispasmodic drugs. These drugs are metoclopramide hydrochloride, hyoscine butyl bromide and tiemonium methyl sulfate. Metoclopramide is usually directed to treat a large variety of epithelial duct disorders [19]. It may be used for the treatment of nausea and physiological reaction [20]. Hyoscine butyl bromide is usually used as associate in spasmolytic treatment for the pain and discomfort iatrogenic by abdominal cramps [21].

Tiemonium methyl sulfate is an antimuscarinic quaternary ammonium agent with peripheral atropine similar effect and is employed within the relief of visceral spasms [22]. The transfer of conjugative plasmids may be affected by these prescribed pharmaceuticals. However, the effect of these non-antibiotic pharmaceuticals on bacterial resistance or antibiotic activity remains unknown. In this work, we study the effect of metoclopramide HCl as a commonly used antiemetic and hyoscine butyl bromide and tiemonium methylsulfate as commonly consumed antispasmodics on conjugal transfer frequency among pathogenic *E. coli* isolates in the GIT.

2. Materials and Methods

2.1 Bacterial isolates and reagents:

Four *E. coli* strains were used for conjugative transfer testing. Two strains were obtained from Hiper® bacterial conjugation kit (HTM004, HiMedia). Donor *E. coli* strain (Product code: TKC181) and Recipient *E. coli* strain (Product Code: TKC182). Two *E. coli* strains were isolated from patients suffering from gastrointestinal tract infection, *E. coli*-1 with plasmid harboring resistance gene for tetracycline was the donor and *E. coli*-5 with high chromosomally encoded resistance to streptomycin was used as the recipient.

Raw materials of streptomycin sulphate (Product code: RM220) and tetracycline hydrochloride (Product code: RM219) were included in Hiper® bacterial conjugation kit (HTM004, HiMedia). Hyoscine butyl bromide, metoclopramide HCl were obtained from Sigma Aldrich (USA) while tiemonium methyl sulfate was purchased from Medica Pharma Specialty Pharmaceutical Chemicals (Netherlands).

2.2 Preparation of isolates for the study:

Strains obtained from Hiper® bacterial conjugation kit, Donor strain A was streaked on 2 LB plates with tetracycline (30µg/mL) and the Recipient strain was streaked on LB plates with streptomycin (100µg/mL) and incubated at 37□C overnight.

For clinical strains, Isolation of *E. coli* strains was performed by MacConkey's agar and confirmed biochemically with various screening tests. Disc method was done to select an *E. coli* isolate (Resistant only to tetracycline) and another one (Resistant only to streptomycin) to be used as donor and recipient respectively. Both genomic and plasmid DNA were extracted from the selected isolates (Qiagen plasmid plus midi kit and Qiaprep spin miniprep kit, USA) and polymerase chain reaction was used to amplify tetracycline and streptomycin resistance genes and *traF* gene (encoded by conjugative plasmid and required for pilus assembly and plasmid transfer). PCR primer sequences and conditions are listed in table (1).

Table (1): PCR conditions and primer sequences

	Primer sequences	PCR conditions	Ref.
traF	Forward: AAGTGTTCAGGGTGCTTCTGC Reverse: GTCGCCTTAACCGTGGTGTT	Initial denaturation (94ºC for 4 min.), 35 cycles (94ºC for 30 sec., 55ºC for 30 sec., 72ºC for 1 min), 7 min final extension	**[11]**
tetA	Forward: GACTATCGTCGCCGCACTTA Reverse: ATAATGGCCTGCTTCTCGCC	Initial denaturation (94ºC for 4 min.), 30 cycles (94ºC for 30 sec., 54ºC for 30 sec., 72ºC for 1 min), 7 min final extension	**[11]**

strA-strB	Forward TATCTGCGATTGGACCCTCTG Reverse CATTGCTCATCATTTGATCGGCT	Initial denaturation (95ºC for 60 s), 30 cycles(95ºC for 60 s, 60ºC for 30 s ,72ºC for 60 s).	**[23]**

2.3 Testing antibacterial activity and MIC detection:

Minimum inhibitory concentrations (MICs) of tetracycline, Streptomycin and non-antibiotic pharmaceuticals were tested using broth microdilution assay. The 96-well plates were incubated at 37 °C for 20 h before the OD600 was measured on the plate reader. Wells containing sterile phosphates buffer saline were used as blank. MICs of the bacterial strains were determined as the concentration of the tested agent which inhibited 90% of the growth. Each bacterial strain under the inhibition of the different agents was tested at least in triplicate Hyoscine butylbromide, metoclopramide HCl and tiemonium methyl sulfate were chosen as the non-antibiotic pharmaceuticals for this study. Concentrations of antibiotics were serially diluted from 8 to 0.03125 µg/ml. Also, non-antibiotic pharmaceuticals were used in therapeutically allowed concentrations [24].

2.4 Conjugative transfer in absence and in presence of non-antibiotics:

2.4.1 Conjugative transfer in absence of non-antibiotics:

A single colony from both donor and recipient strains was inoculated in 6ml of LB broth. Then, incubated at 37□C overnight. One ml of overnight grown culture of standard strains or clinical strains was added to 25 ml LB broth with tetracycline. Three ml of overnight culture of the tested strains were added to 25 ml of LB with streptomycin. Cultures were overnight incubated at 37□C in a shaker. Two hundred µL of each donor and recipient cultures were added to a sterile test tube labeled as conjugated sample after gentle mixing and incubated for 1.5 hr without shaking. Two hundred µL of respective cultures were added to tubes labeled as donor and recipient with gentle mixing and incubated for 1.5hr without shaking. Then, 0.1 ml of each culture was plated on

antibiotic containing culture media (one plate containing tetracycline, another plate containing streptomycin and the third containing both tetracycline and streptomycin to isolate transconjugants). Transconjugant colonies' number was divided by donor colonies' number to calculate the conjugative transfer frequency. The value of frequency of conjugal transfer was the average of four different trials. Growth of donor, recipient and transconjugants was not included in calculation due to absence of provided nutrients during the conjugation process [25].

2.4.2 Conjugative transfer in the presence of non-antibiotics:

This work designed a mating model to detect the effect of non-antibiotic pharmaceuticals on conjugative transfer between the tested *E. coli* strains. This was achieved by growing donor and recipient isolates to an OD of 1.8 at 600 nm and mixing donor and recipient in nutrient broth (1:2) ratio in the presence of the following sub-inhibitory concentrations: 0.265 μg/ml (Equivalent to plasma concentration) , 0.53 μg/ml, 1.06 μg/ml, 2.12 μg/ml and 4.24 μg/ml for metoclopramide HCl. For hyoscine butyl bromide; 0.005 μg/ml (Equivalent to plasma concentration), 0.01 μg/ml, 0.02 μg/ml, 0.04 μg/ml and 0.08 μg/ml were used. Finally, tiemonium methyl sulfate has sub-inhibitory concentrations of 0.8 μg/ml (plasma concentration), 1.6 μg/ml, 3.2 μg/ml, 6.4 μg/ml and 12.8 μg/ml. Levofloxacin was used at its sub-MIC as a positive control for conjugative transfer (0.125 μg/ml) [26]. After incubation for 3 hr. at 37ºC, mixture of donor and recipient (20 μL) was plated on tetracycline-streptomycin nutrient agar plates to count colonies of transconjugants. Transconjugant colonies' number was divided by donor colonies' number to calculate the conjugative transfer frequency. The value of frequency of conjugal transfer was the average of four different trials **[11]**.

2.5 Reverse conjugation experiment in the presence of non-antibiotic agents:

Transconjugant bacteria were considered as donor cells. Standard and clinical recipients described previously were used for the conjugate mating system. All strains were tested using different concentrations of the tested agents. Then, mixed by vertexing and incubated for 3h at 37□C without shaking. New transconjugants were used to inoculate agar plates containing antibiotics. Colonies were counted and the frequency of transfer was determined as prescribed above [11].

2.6 Verification of conjugative plasmid transfer:

- **2.6.1 Determination of Minimum inhibitory concentration of transconjugants:**

Minimum inhibitory concentrations (MICs) of transconjugants against tetracycline and streptomycin were determined by broth microdilution methods. The resulting MICs of transconjugants were compared to donor and recipient cells' MICs [24]**.**

- **2.6.2 Detection of plasmids:**

Colonies of transconjugants on selective tetracycline-streptomycin agar plates were picked and stored at -80ºC in glycerol stock (30%). Qiagen plasmid plus midi kit (Qiagen, USA) was applied for extraction of plasmids from donor and transconjugants. PCR was used to amplify plasmid-encoded *traF* and *tetA* genes in both donor and transconjugant. Agarose gel electrophoresis (1%) was used to observe plasmids and amplicons [25].

- **2.7 Transmission Electron Microscopy (TEM):**

Effect of non-antibiotic pharmaceuticals on conjugative activity was studied using TEM (JEM1010, JEOL, Tokyo, Japan). After performing conjugation experiment with non-antibiotic pharmaceuticals and incubation for 24 hr, samples for TEM were collected and imaged. Also, a control sample in absence of non-antibiotics was included. Samples were prepared for TEM according to standard guidelines **[27]**.

2.8 Statistical analysis

Statistical analysis was performed using GraphPad version 8.3.0 software. All data were obtained from at least three biological replicates and presented as mean ± SD. Unpaired *t*-test (normally distributed data) between two groups or one-way ANOVA among multiple groups were used to calculate *P*-values. Differences with $P < 0.05$ were considered significant [28].

3. Results

3.1 Antibacterial activity of non-antibiotic pharmaceuticals:

Broth microdilution assay illustrated no inhibitory activity for metoclopramide, hyoscine butyl bromide and tiemonium methyl sulfate (MIC > 1024 μg/ml). Agar well diffusion assay was used to confirm the previous findings by reporting no inhibitory activity for the non-antibiotic pharmaceuticals against donor and recipient isolates (Figure 1).

Figure 1: Antibacterial activity of non-antibiotic pharmaceuticals: A) against donor isolate, B) against recipient isolate.

3.2 Non-antibiotic pharmaceuticals have different effects against ARGs conjugative transfer:

For both standard and clinical *E. coli* strains; it was found that for the addition of non-antibiotic pharmaceuticals, metoclopramide HCl, at all five concentrations (from 0.265 to 4.24 μg/ml), the number of transconjugants dramatically increased ($P < 0.05$). Hyoscine butyl bromide, at concentrations from 0.005 to 0.01 μg/ml, slightly increased transconjugant number ($P < 0.05$). In contrast, tiemonium methyl sulfate at concentrations from 0.8 to 12.8 μg/ml, decreased the transconjugant number ($P < 0.05$).

Fig. 2a illustrates transconjugant colonies in absence of antibiotics and non-antibiotics as a control. Efficiency of conjugation between donor and recipient isolates was greatly promoted by metoclopramide HCl (Fig. 2b) while was dramatically decreased by hyoscine butylbromide (Fig. 2c). Also, slight induction of conjugal transfer by tiemonium methylsulfate was observed (Fig. 2d). Levofloxacin at sub-MIC level greatly increased conjugal transfer compared to control conjugation tube (Fig 2e). Transfer ratio (expressed as number of transconjugants per recipient cells) and fold change of transfer ratio were illustrated for the tested non-antibiotic agents at different concentrations and levofloxacin at sub-MIC (Table 2). Fold change of transfer ratio was greatly enhanced in presence of metoclopramide HCl (Figure 3) and slightly increased with tiemonium methylsulfate (figure 4). However, it was decreased in presence of hyoscine butylbromide as compared to negative control (Figure 5).

Figure (2): Effect of antibiotics and non-antibiotic pharmaceuticals on conjugal transfer frequency: A) Control, B) Metoclopramide HCl, C) Hyoscine butylbromide D) Tiemonium methylsulfate, E) Levofloxacin at sub-MIC

Table (2): Conjugative transfer under the exposure of different concentrations of non-antibiotic pharmaceuticals

	Number of recipients in 1 ml	Absolute number of transconjugants in 1 ml	Fold change of transconjugant absolute number	Transfer ratio (number of transconjugant / number of recipient)	Fold change of Transfer ratio
Control	$2.5 \times 10^4 \pm 817$	74±5.72	1.00±0.08	$29.7 \times 10^{-4} \pm 3 \times 10^{-4}$	1.00±0.11
Metoclopramide HCl					
0.265 µg/ml	$2.3 \times 10^4 \pm 12415$	485±8.16	6.55±0.4	$195 \times 10^{-4} \pm 16 \times 10^{-4}$	6.55±0.16*
0.53 µg/ml	$2.4 \times 10^4 \pm 12034$	523±16.33	7.06±0.33	$238 \times 10^{-4} \pm 31 \times 10^{-4}$	8.2±1.5*
1.06 µg/ml	$2.2 \times 10^4 \pm 11002$	578±81.65	7.81±0.51	$240 \times 10^{-4} \pm 23 \times 10^{-4}$	7.97±0.2*
2.12 µg/ml	$2.5 \times 10^4 \pm 12472$	634±27.76	8.57±1.05	$255 \times 10^{-4} \pm 14 \times 10^{-4}$	8.67±0.5*
4.24 µg/ml	$2.4 \times 10^4 \pm 11885$	670±81.65	9.05±1.82	$280 \times 10^{-4} \pm 41 \times 10^{-4}$	9.73±2.4*
Hyoscine butylbromide					
0.005 µg/ml	$2.5 \times 10^4 \pm 817$	39±16.33	0.53±0.18	$16 \times 10^{-4} \pm 7 \times 10^{-4}$	0.51±0.18*
0.01 µg/ml	$2.5 \times 10^4 \pm 4082$	32±8.16	0.43±0.08	$14 \times 10^{-4} \pm 6 \times 10^{-4}$	0.44±0.14*
0.02 µg/ml	$2.4 \times 10^4 \pm 816$	28±12.25	0.38±0.2	$12 \times 10^{-4} \pm 5 \times 10^{-4}$	0.42±0.23*
0.04 µg/ml	$2.2 \times 10^4 \pm 3266$	19±4.08	0.26±0.08	$9 \times 10^{-4} \pm 3 \times 10^{-4}$	0.32±0.14*
0.08 µg/ml	$2.4 \times 10^4 \pm 4082$	15±5.72	0.20±0.06	$7 \times 10^{-4} \pm 3 \times 10^{-4}$	0.22±0.08*
Tiemonium methylsulfate					
0.8 µg/ml	$2.5 \times 10^4 \pm 2449$	74±8.16	1.00±0.19	$29.6 \times 10^{-4} \pm 4 \times 10^{-4}$	1.01±0.12
1.6 µg/ml	$2.5 \times 10^4 \pm 3266$	78±14.7	1.05±0.28	$31 \times 10^{-4} \pm 2 \times 10^{-4}$	1.06±0.18
3.2 µg/ml	$2.3 \times 10^4 \pm 5312$	83±10.6	1.12±0.23	$37 \times 10^{-4} \pm 8 \times 10^{-4}$	1.26±0.3
6.4 µg/ml	$2.5 \times 10^4 \pm 4899$	87±5.72	1.18±0.17	$37 \times 10^{-4} \pm 5 \times 10^{-4}$	1.24±0.05
12.8 µg/ml	$2.4 \times 10^4 \pm 6532$	88±13.06	1.19±0.27	$40 \times 10^{-4} \pm 17 \times 10^{-4}$	1.42±0.74
Levofloxacin at sub-MIC (0.125 µg/ml)	$2.2 \times 10^4 \pm 1633$	2500±408	33.78±8.21	$1150 \times 10^{-4} \pm 253 \times 10^{-4}$	40.08±13.2*

Results are shown as mean ± SD, significant differences between non-antibiotic dosed samples and the control were analyzed by independent-sample *t* test.* $P < 0.05$ were considered significant.

Figure (3): Fold changes of transfer ratio under the exposure of metoclopramide HCl at different concentrations

Figure (4): Fold changes of transfer ratio under the exposure of tiemonium methyl sulfate at different concentrations

Figure (5): Fold changes of transfer ratio under the exposure of hyoscine butyl bromide at different concentrations.

3.3 Reverse conjugation experiment in the presence of non-antibiotic agents:

To test if the transconjugant is transferable, the reverse conjugative experiment is performed. The plasmid is transferred from conjugants to donners. It was seen that the conjugative transfer frequency was enhanced in the presence of metoclopramide HCl as compared to negative control. Also, an increases in the fold changes of the transfer frequency occurred in the presence of the tested drug. Transconjugant colonies were chosen and re-grown on selection plates to verify the identity of the recipient cells.

3.4 Determination of Minimum inhibitory concentration of transconjugants:

The MICs of the transconjugants against tetracycline and streptomycin were detected (Table 3) and were the same or higher than the donor or recipients MICs.

Table (3): Minimum inhibitory concentrations (MICs) of donor, recipient, and different transconjugants towards antibiotics

Antibiotics	MICs (mg/L)				
	Donor	Recipient	TC 1	TC 2	TC 3
Tetracycline	32	4	32	32	32
Streptomycin	4	64	64	64	64

*TC 1-3: transconjugants in mating system treated with hyoscine butylbromide, metoclopramide HCl and tiemonium methyl sulfate, respectively.

3.5 Detection of plasmids:

Confirmation of successful conjugal transfer was done by plasmid analysis on agarose gel electrophoresis. Conjugative plasmid was detected in donor and transconjugant strains as illustrated by first and second lanes (Figure 6). Recipient isolate did not show any plasmid as recorded in lane 3.

Figure (6): Plasmid detection in donor (lane 1), transconjugants (lane 2) and recipient (lane 3)

3.6 Effect of non-antibiotic pharmaceuticals on conjugation efficiency:

Figure 4 illustrates TEM images of conjugative transfer in the presence of non-antibiotic pharmaceuticals and in their absence as a control (Fig. 7A1,2). Metoclopramide HCl induced conjugal transfer between donor and recipient as seen in the close contact between cells, periplasmic bridges and aggregation of cells (Fig. 7B1,2). Hyoscine butylbromide decreased conjugal activity as observed in the little affinity between cells (Fig. 7C).

Figure (7): TEM images for conjugal activity between donor and recipient A) Control, B) In presence of metoclopramide HCl, C) In presence of hyoscine butyl bromide

4. Discussion

The present findings highlight the effect of non-antibiotic pharmaceuticals on bacterial behavior and how this will influence the dissemination of antibiotic resistance between isolates changing the duration of activity of used antibiotics. Antibiotic resistance is considered a major health problem which arose mainly due to the misuse of antimicrobial agents all over the world. This dilemma is usually attributed to various causes such as: availability of antibiotics everywhere and health care persons may suffer from lack of education. Also, many patients stop treatment with antibiotics upon feeling better, use leftover antibiotics and may use antibiotics in treating viral infections [29-31]. The rapid dissemination of antibiotic resistance genes (ARGs) among bacterial species occurs through horizontal gene transfer (HGT). Conjugation is a common mechanism by which bacteria horizontally transfer their resistance genes for adaptation with stress conditions such as continuous antibiotic exposure and lack of nutrients **[32]**. Bacterial conjugation is a major promoter for bacterial genome evolution which supply bacteria by required genes for antibiotic resistance, biofilm formation, virulence and heavy metal resistance **[33, 34]**. Sub-inhibitory concentration of antibiotics has been found to induce dissemination of antibiotic resistance by HGT particularly through conjugation and transformation **[11, 35].** Not only antibiotics affect gene transfer between bacteria but also non-antibiotic pharmaceuticals may be supposed to have an effect as recorded for more than 200 non-antibiotics which influence gut bacteria like antibiotics do **[15]**. All over the world, non-antibiotic pharmaceuticals represent 95% of the total drugs in the market. Although their wide distribution, little is known about how non-antibiotic pharmaceuticals affect HGT between bacteria **[36, 37]**. In the present work, effect of metoclopramide HCl, hyoscine butylbromide and tiemonium methylsulfate on conjugal transfer between *E. coli* isolates was studied. Also, levofloxacin at sub-inihibitory concentration was included. Hyoscine butylbromide and tiemonium methylsulfate have antispasmodic activity while metoclopramide HCl has antiemetic effect. Metoclopramide HCl has antibiotic-like effect behavior as for promoting conjugal transfer between isolates especially when antibiotic is administered at sub-inhibitory concentration. While conjugal transfer frequency is dramatically increased by metoclopramide HCl, it was obviously suppressed by hyoscine butylbromide. This means that co-administration of

metoclopramide HCl as antiemetic with antibiotics may promote the transfer of antibiotic resistance genes among pathogenic bacteria in the GIT decreasing the susceptibility to the antibiotic in the long run use. Acceleration of dissemination of antibiotic resistance was reported for non-antibiotic pharmaceuticals such as ibuprofen, naproxen, diclofenac, gemfiprozil and propranolol as plasmid-borne conjugal transfer was promoted between bacteria of the same and different genera **[11]**. Also, dissemination of plasmid-mediated antibiotic resistance was induced by the antiepileptic carbamazepine **[38]**. These findings agree with what was obtained in our study for metoclopramide HCl. Norepinephrine has not revealed any effect on plasmid conjugal transfer between E.coli isolates **[39]**. The previous result agrees with what achieved in the present work for tiemonium methylsulfate which caused a slight increase in conjugal transfer between *E. coli* isolates. In the present study, hyoscine butylbromide dramatically decreased conjugal transfer between tested *E. coli* supporting the activity of co-administered antibiotics by weakening the resistance of targeted pathogenic bacteria. Expired drug metoclopramide HCl indicated antibacterial activity for carbon steel in 0.5 M H3PO4 solution **[40]** which disagrees with what achieved in the present work as none of the tested non-antibiotics revealed antibacterial activity against isolates. TEM imaging was applied to reveal changes of cell arrangement as an indicator to study the effect of metoclopramide HCl and hyoscine butylbromide on bacterial cells. Aggregation of cells, formation of periplasmic bridges and decreasing distances between cells were obviously noticed with metoclopramide HCl while with hyoscine butylbromide little affinity between cells was observed. In absence of non-antibiotic pharmaceuticals, cells were separated and intact but in presence of non-antibiotics, cells became closer to each other with partially damaged cell membrane **[11]**. In the present findings, the role of non-antibiotic pharmaceuticals in dissemination of antibiotic resistance between pathogenic gastrointestinal tract *E. coli* isolates was clearly demonstrated. Previous findings turn on alarm for bacterial response towards different administered pharmaceuticals highlighting the bacterial behavior when non-antibiotics are co-administered with antibiotics. More work is required to illustrate the effect of pharmaceuticals in the market on the transfer of resistance genes and how this would contribute to maximize or minimize the antibiotic resistance crisis.

5. Conclusion

In prescribing antibiotics for treating intestinal pathogenic *E.coli*, antiemetic metoclopramide HCl was found to increase the spread of resistance against the co-administered antibiotic by promoting the plasmid-mediated conjugal transfer of antibiotic resistance genes followed by tiemonium methylsulfate . While, antispasmodic hyoscine butylbromide showed lower rate of conjugal transfer in comparison to contro

Author contribution

Formal analysis, Alaa Alqahtani and Eman Farouk; Investigation, Sahar Ghanem and Eman Farouk; Methodology, Doaa Safwat Mohamed, Mohamed El-Mokhtar, Sahar Ghanem, Ramadan Yahia, Mohammed A. S. Abourehab and Eman Farouk; Project administration, Doaa Safwat Mohamed; Software, Mohammed A. S. Abourehab; Supervision, Rehab Mahmoud Abd El-Baky and Alaa Alqahtani; Validation, Doaa Safwat Mohamed and Mohamed El-Mokhtar; Visualization, Mohamed El-Mokhtar and Ramadan Yahia; Writing – original draft, Rehab Mahmoud Abd El-Baky, Sahar Ghanem, Ramadan Yahia, Alaa Alqahtani, Mohammed A. S. Abourehab and Eman Farouk.

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Institutional Review Board Statement The study was conducted according to the guidelines of the Declaration of Helsinki, priori approval (No. 2/2022) by the ethical committee of Faculty of Pharmacy, Deraya University.

Informed Consent Statement

Not applicable.

Data Availability Statement

Not applicable.

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Conflicts of Interest

The authors declare no conflict of interest.

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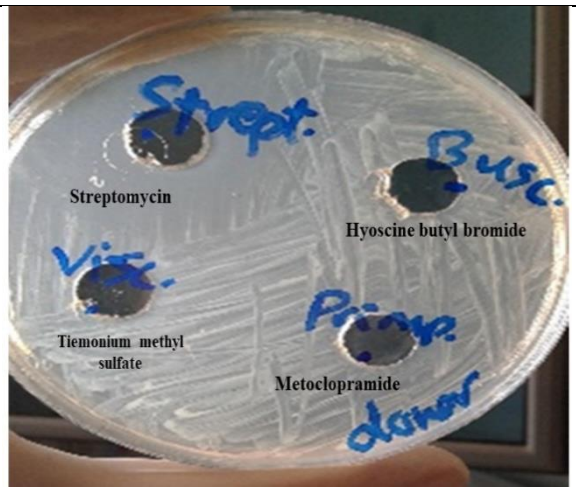
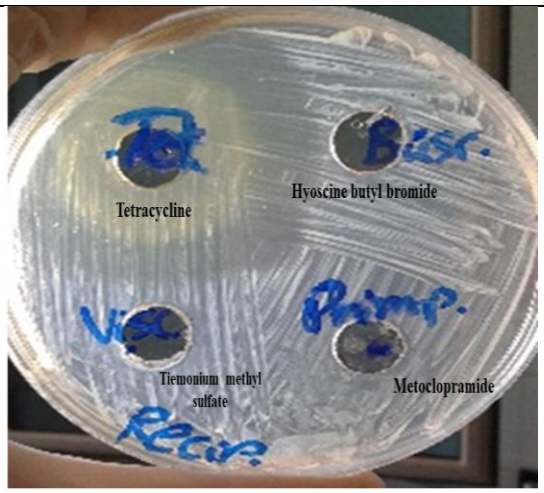
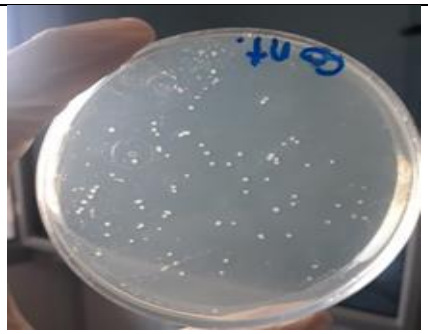


A



B

Figure 1: Antibacterial activity of non-antibiotic pharmaceuticals: A) against donor isolate, B) against recipient isolate.



A

B

C

D

E

Figure (2): Effect of antibiotics and non-antibiotic pharmaceuticals on conjugal transfer frequency: A) Control, B) Metoclopramide HCl, C) Hyoscine butylbromide D) Tiemonium methylsulfate, E) Levofloxacin at sub-MIC

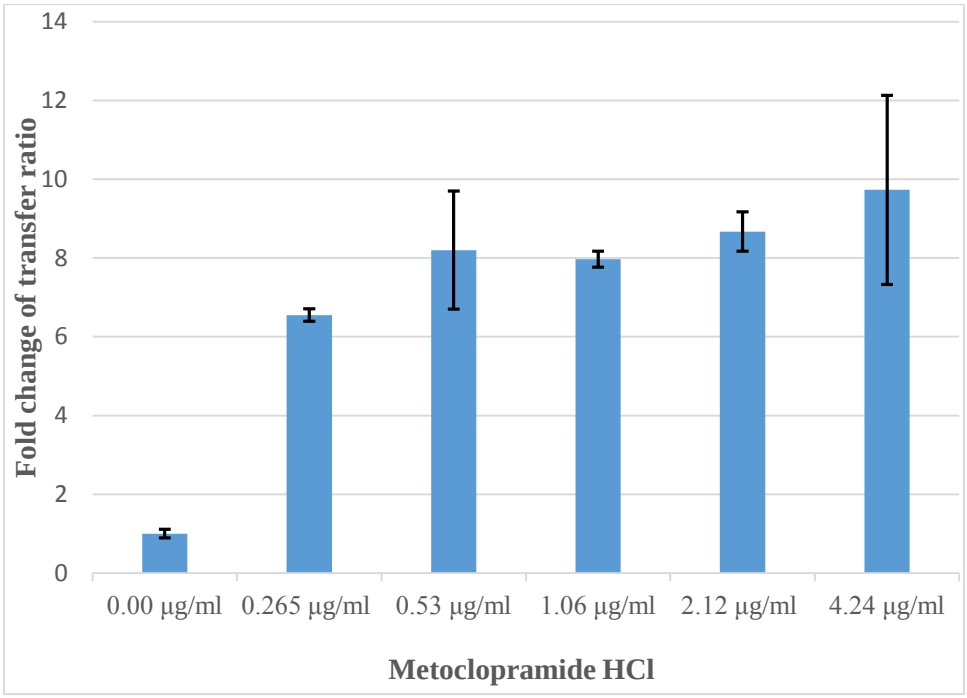


Figure (3): Fold changes of transfer ratio under the exposure of metoclopramide HCl at different concentrations

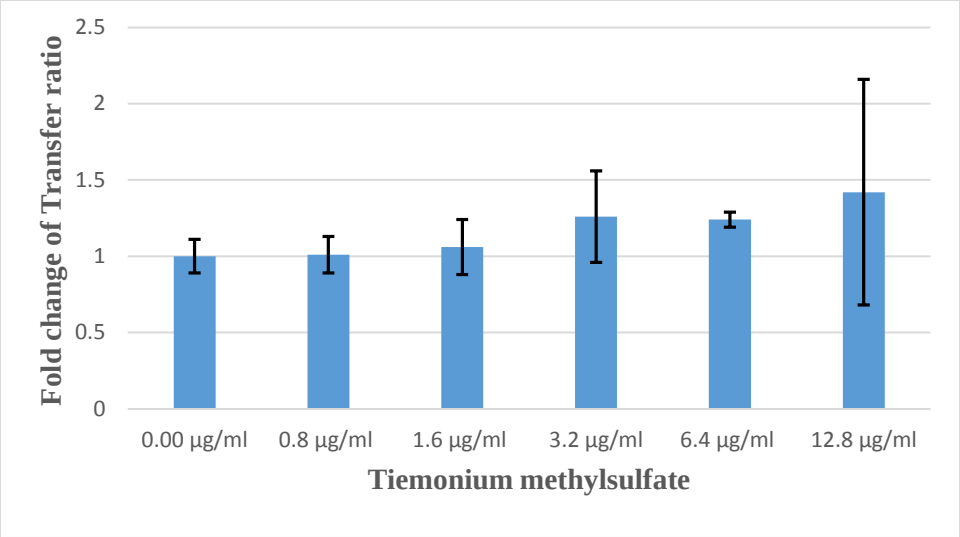


Figure (4): Fold changes of transfer ratio under the exposure of tiemonium methyl sulfate at different concentrations

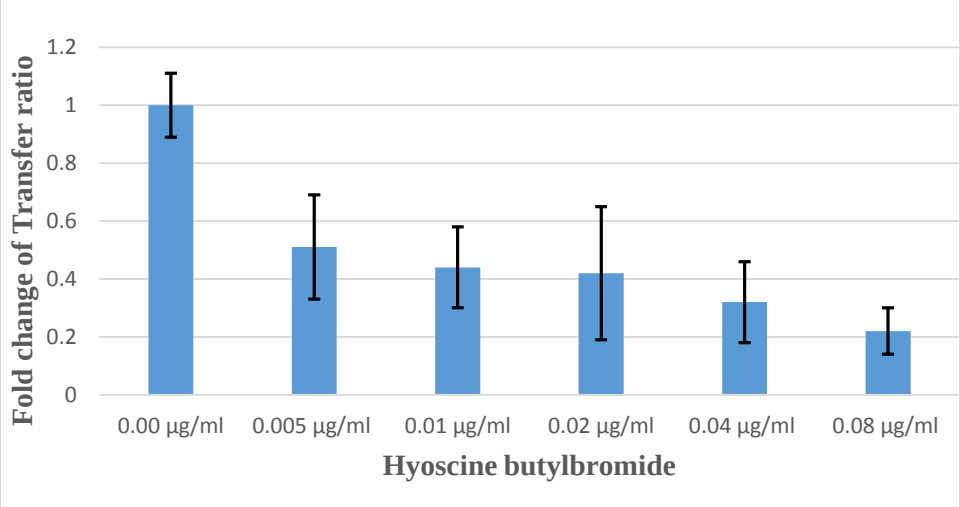


Figure (5): Fold changes of transfer ratio under the exposure of hyoscine butyl bromide at different concentrations

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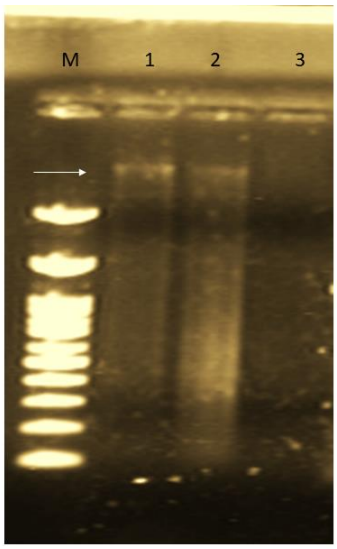
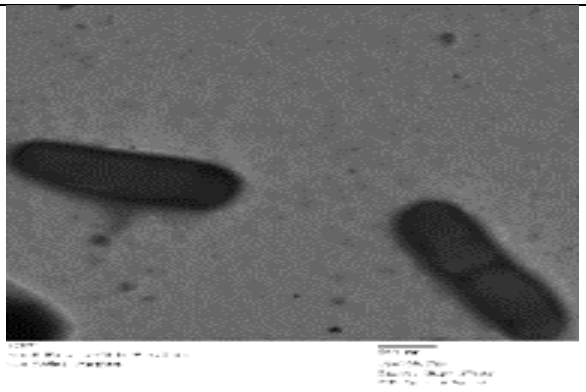
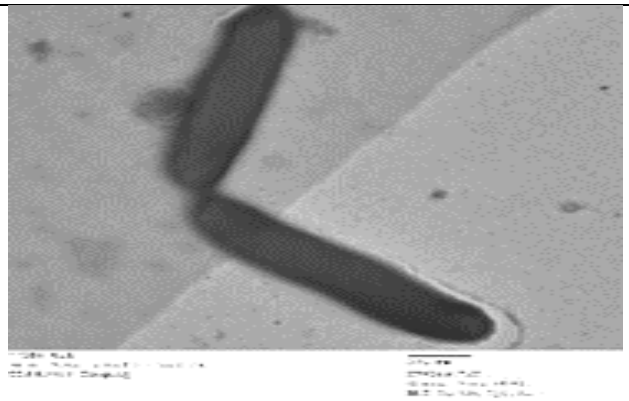
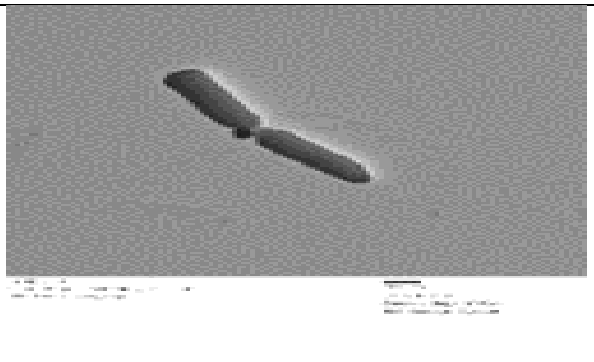
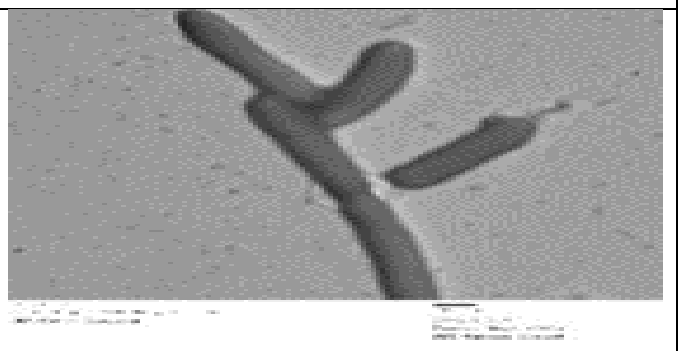


Figure (6): Plasmid detection in donor (lane 1), transconjugants (lane 2) and recipient (lane 3)

A		
B		

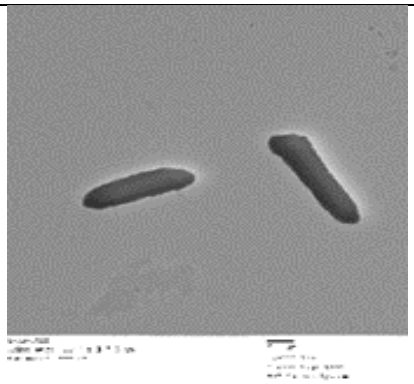
C	

Figure (7): TEM images for conjugal activity between donor and recipient A) Control, B) In presence of metoclopramide HCl, C) In presence of hyoscine butyl bromide