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Role of Omeprazole in Enhancement of Antibiotic Resistance in *E. coli*

S. A. Al-Bakri, R. R. Jabri, E. A. Ajeel

Department of Biotechnology, School of Applied Science, University of Technology.

Abstract

E. coli was isolated, and it was Gram-negative rod bacteria that was colony circular, regular edged, thick somewhat glitter and viscous(less). It was lactose fermenter bacteria and belongs the family of *Enterobacteriaceae*.

E. coli showed sensitivity to all used antibiotics except Erythromycin (E), Cloxacellin (CX), Rifampin (RA), Cephalothin (KF), Ampicillin (AM), and Penicillin (P).

The experimental results of antibiotic sensitivity of *E. coli* in media containing different concentrations of omeprazole, a proton pump inhibitor, showed an enhancement of resistance by decreasing the sensitivity of *E. coli* inversely with drug concentration against the antibiotics that *E. coli* was sensitive to.

It seems that omeprazole changed cell membrane potential of *E. coli* which led to depolarization of cell membrane as a result of inhibition of the proton pump mechanism. This made the bacterial cell not willing to uptake antibiotics.

Introduction

Escherichia coli is a member of the genus *Escherichia* that includes in the family of *Enterobacteriaceae* [1]. It is a Gram-negative, nonsporing, facultative rod that ferments lactose with gas formation within 48 hour, at 35°C [2]. *E. coli* is the best studied bacterium and the experimental organism of choice for many microbiologists. It is a major inhabitant of the colon of human and other worm-blooded animals[3,4]. It is the major causative agent of urinary tract infection (UTI). UTI is treated by antimicrobial drugs that destroy pathogenic microorganisms at low concentration called Minimum Lethal Concentration (MLC) or inhibit their growth at low concentration called Minimum Inhibitory Concentration (MIC) [5,6].

Over the past several years, the medical community has become increasingly concerned over the ability of certain bacteria to develop resistance to antibiotics [7,8]. Accordingly, there is a danger of losing the battle against certain pathogens (disease causing organisms) by using the antibiotics in the treatment[7]. Bacteria do not become resistant to antibiotics without presence of mechanisms by which resistance may be conferred [9,10]. There are four main mechanisms by which microorganisms can exhibit resistance to antimicrobials which: drug inactivation or modification, alteration of target site, alteration of metabolic pathway, or reducing drug accumulation [7]. Furthermore, there are several means through which the uptake of a drug into a cell can be reduced: changes in the structure of the cell membrane, loss, or mutations of porins in the cell membranes, and active efflux of the drug from the cell [11,12].

The drug efflux systems are membrane transport proteins. Proton motive force (pmf) system is one of the drug efflux mechanisms, is principally found in bacteria and yeasts. The pmf is also known as the electrochemical proton gradient, and a chemical proton gradient. It functions as antiporter, and therefore mediates drug efflux in exchange with proton translocation into the cell [13].

Current study dealt with the role of proton motive force mechanism in drug efflux pump in *E.coli*, a bacterial model, by using omeprazole, an inhibitor of pmf mechanism. Omeprazole is one of the most widely prescribed drugs internationally and is used in the

treatment of dyspepsia, peptic ulcer disease (PUD), gastroesophageal reflux disease (GORD/GERD) and Zollinger-Ellison syndrome [14].

Materials and Methods

Isolation of *E. coli*: An *E. coli* was isolated from patient with UTI, cutivated in Macconkey agar (HIMEDIA) plate, and then cultivated in Nutrient agar (HIMEDIA), and then its shape was identified [15].

Antibiotic Sensitivity (Disk Diffusion Method): Antibiotic sensitivity for isolated bacteria was done by using a combination of antibiotics disks (Bioanalyse); including Cloxacellin (CX) 1µg, Erythromycin (E) 15µg, Tetracycline (TE) 30µg, Ampicillin (AM) 10µg, Gentamicin (CN) 10µg, Nalidixic Acid (NA) 30µg, Cephalothin (KF) 30µg, Neomycin (N) 30µg, Penicillin (P) 10µg, Lincomycin (L) 2µg, Cephotaxime (CTX) 30µg, Trimethoprime 1.25µg + Sulfamethorazole 23.75µg (SXT) 25 µg, Chloramphenicol (C) 30µg, Nitrofurantion (F) 300µg, Rifampin (RA) 5µg, and Tobramycin (TOB) 10µg. These disks were stored at 4°C using disk diffusion method as the following: 0.1ml of each strain was cultured by spreading on the suface of nutrient agar plate, and then antibiotic disks were placed on the surface of agar using sterile forceps and incubated at 37°C for 24 hr. The results were indicated according to formation of inhibition zone around the disk for sensitive or not formation of inhibition zone around the disk for resistance. [16].

Sensitivity to Omeprazole: Two different concentrations of omeprazole (stock solution of omeprazole of 1mg/ml. was prepared by dissolving the granules of 1 capsule [20mg] of omeprazole [Ajanta] in 20ml of distilled water and stored at -20°C) were prepared (100 and 300 µg/ml), and each concentration was added to 20ml of Nutrient agar and left to solidify, and then *E. coli* was cultivated by streaking, and left to incubate at 37°C for 24 hr. Then the sensitivity of *E.coli* to omeprazole different concentrations was detected. [17,18].

Testing the Role of Omeprazole in Antibiotic Sensitivity: Each of the prepared concentrations of omeprazole (100 and 300 µg/ml) was added to 20 ml of Nutrient agar and left to solidify. *E. coli* was inoculated on the surface of each plates of Nutrieint agar (the plates that contain omeprazole). The antibiotic disks (same antibiotic disks that were used in antibiotic sensitivity test) were added to the surface of Nutrient agar of each plate. Plates were incubated at 37°C for 24 hour. The results were detected and recorded according to formation, absence, increasing, or decreasing of inhibition zone around the disk .[17,18].

Results and Discussion

Isolation of E. coli: *E. coli* was purified by cultivation on MacConkey agar media, the morphological characteristics and properties of *E. coli* were determined as: circular, regular edge, thick somewhat, glitter pink, viscous, and lactose fermenter [2].

Antibiotic Sensitivity: The sensitivity to antibiotics was determined and the results indicated that *E. coli* was sensitive to 10 antibiotics and resistant to 6 antibiotics from 16 types of antibiotics used (Table 1). These results were used as a control for further comparison (Table 3).

Sensitivity to Omeprazole: The recommended dosages for patients taking omeprazole are 10mg, 20mg, or 40mg. But the most frequent side effects of omeprazole are headache, diarrhea, abdominal pain, nausea, dizziness, trouble awakening and sleep deprivation [19]. Omeprazole may be associated with a greater risk of hip fractures [20], *Clostridium difficile* diarrhea, and heart problems, including cardiac arrest [21].

The study dealt with minimum concentrations (100, and 300 µg/ml) (MIC) of omeprazole to minimize its side effect on human and ensure its activity on E. coli. (17,18). *E. coli* was cultivated in each concentration of omeprazole containing agars after 24 hr. of incubation at 37°C. The study found that *E. coli* grew at both omeprazole concentrations (Table 2) and this drug showed no killing effect on *E. coli* [22,23]. For this reason this

research used omeprazole to find out its role in modulation of antibiotic resistance in *E. coli*. Since omeprazole is a proton pump inhibitor [13,22].

Omeprazole - Antibiotic Sensitivity Test: Results in (Table 3) illustrate the antibiotic sensitivity of *E. coli* in petridishs containing two concentrations of omeprazole; 100 µg/m and 300 µg/ml. There were obvious decreasing in the diameters of inhibition zones of the most antibiotic types that *E. coli* was sensitive to reversely with omeprazole concentrations as compared with control group in (Table 3). In addition, *E. coli* exhibited its resistance against TE and F at 300µg/ml of omeprazole. Accordingly, the total antibiotic types that E. coli was resistant to, had increased at 300µg/ml of omeprazole containing medium to become 8 from 16 antibiotic types (Table 3).

These results proposed that omeprazole had an important role in the enhancement of resistance of *E. coli* against antibiotics [24]. Omeprazole is a proton pump inhibitor [13,22]. The proton pump system is an integral membrane protein that is capable of grabbing protons from the matrix (the space enclosed by the two membranes) and releasing the protons into the inter-membrane space. The confined protons create a difference or gradient in both pH and electric charge and establish an electrochemical potential. Because there's a higher concentration of protons in the inter-membrane space compared to inside the cell, there's pressure to return protons down the concentration gradient to restore the balance. This pressure is called the proton motive force (pmf) [25].

In current study, after treatment of E. coli with omeprazole, a gradient in electric charge might be created, the concentration of protons inside the bacterial cell was higher than its concentration in the inter-membrane space [26]. There was no way to restore the balance by pumping the protons outside the cell [27] due to the action of omeprazole as a proton pump inhibitor. Blocking the passage of proton pumping seemed to create a decreasing in cellular membrane potential (Depolarization of cell membrane) [28]. Depolarization of cell membrane may make *E. coli* not willing to uptake the antibiotics into the cell [29]The results of antibiotic sensitivity in omeprazole containing media give an indirect suggestion that there were no effect of gradient in pH [30] on the downsizing of porins to make the permeability of cell membrane selectable [31] for the antibiotics of low molecular weight [32]. This suggestion depends on that TE and F don't have larger molecular weight than the other antibiotics (Table 4) to let *E. coli* showed resistance or decreasing the sensitivity against them after the treatment with 100 µg/ml omeprazole as found in (Table 3), while the antibiotic sensitivity has been decreased against the other types of antibiotics at 100 µg/ml and 300 µg/ml omeprazole containing media.

These results are in agreement with the study of Perlin, and his colleagues [24]. They reported the electrogenic behavior of proton transport by the H^+-ATPase in *Saccharomyces cerevisiae*. H^+-ATPase is encoded by *pma1* gene. The study found that mutations within *pma1* may alter steady-state membrane potential formation, possibly through a change in the electrogenicity of the H^+-ATPase. This make the mutant exhibited its resistance to hygromycin B that may be mediated via depolarization of the cellular membrane potential.

Conclusion

The experimental results revealed that the treatment of *E. coli* with omeprazole, a proton pump inhibitor, did not modulate the resistance phenomenon in *E. coli* but it enhanced the resistance by decreasing the sensitivity of E. coli inversely with the drug concentration against the antibiotics that *E. coli* was sensitive to (before the treatment with omeprazole).

The study suggested that the decreasing in the sensitivity may mediate by depolarization of cellular membrane via a potential change in cell membrane as a result of proton pump inhibition by omeprazole.

Accordingly, the modulation of antibiotic resistance by a proton pump inhibitor is not recommended because the treatment of bacterial infection may become more complicated.

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IBN AL- HAITHAM J. FOR PURE & APPL. SCI. VOL.23 (3) 2010

Table 1. Susceptibility of *E. coli* to several antibiotics

Antibiotic	*E. coli* Susceptibility Diameter/mm
E	0
CX	0
RA	0
KF	0
AM	0
P	0
F	6
TE	11
SXT	20
CTX	20
TOB	15
L	10
C	23
N	26
NA	26
CN	24

0 = Resistant, Erythromycin (E), Cloxacellin (CX) , Rifampin (RA), Cephalothin (KF), Ampicillin (AM), Penicillin (P), Nitrofurantion (F), Tetracycline (TE), Trimethoprime + Sulfamethorazole (SXT), Cephotaxime (CTX), Tobramycin (TOB), Lincomycin (L), Chloramphenicol (C), Neomycin (N), Nalidixic Acid (NA), Gentamicin (CN).

Table 2. Susceptibility of *E. coli* to omeprazole

Omeprazole µg/ml	***E. coli* Growth**
100	+
300	+

+ = Growth.

IBN AL- HAITHAM J. FOR PURE & APPL. SCI. VOL.23 (3) 2010

Table 3. Susceptibility of *E. coli* to several antibiotics in omeprazole containing medium

Antibiotic	*E. coli* Susceptibility Diameter/mm		
	Control	Omeprazole -µg/ml	
		100	300
E	0	0	0
CX	0	0	0
RA	0	0	0
KF	0	0	0
AM	0	0	0
P	0	0	0
F	6	6	0
TE	11	7	0
SXT	20	15	15
CTX	20	15	10
TOB	15	15	9
L	10	11	9
C	23	15	11
N	26	18	10
NA	26	28	26
CN	24	20	9

0 = Resistant, Erythromycin (E), Cloxacellin (CX) , Rifampin (RA), Cephalothin (KF), Ampicillin (AM), Penicillin (P), Nitrofurantion (F), Tetracycline (TE), Trimethoprime + Sulfamethorazole (SXT), Cephotaxime (CTX), Tobramycin (TOB), Lincomycin (L), Chloramphenicol (C), Neomycin (N), Nalidixic Acid (NA), Gentamicin (CN).

Table 4. Molecular weight of used antibiotics (en.wikipedia.org)

Antibiotic	Molecular Weight g/mol
RA	882.940
E	733.930
N	614.644
SXT	543.599
CN	477.596
TOB	467.515
CTX	455.470
TE	444.435
CX	435.880
L	406.538
KF	396.440
P	350.391
AM	349.406
C	323.132
F	238.160
NA	232.235

Rifampin (RA), Erythromycin (E), Neomycin (N), Trimethoprime + Sulfamethorazole (SXT), Gentamicin (CN), Tobramycin (TOB), Cephotaxime (CTX), Tetracycline (TE), Cloxacellin (CX) , Lincomycin (L), Cephalothin (KF), Penicillin (P), Ampicillin (AM), Chloramphenicol (C), Nitrofurantion (F), Nalidixic Acid (NA).

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دورالأومبرازول في تعزيز مقاومةِ المضادات الحياتية في *E. coli*

صالح عبد الرضاالبكري، ريا رعد جبرائيل جبري، أسراء عطية عجيل

فرع التقنيات الأحيائية، قسم العلوم التطبيقية، الجامعة التكنولوجيه

الخلاصة

عزلت بكتريا *E.coli* بالأعتماد على صفاتها التشخيصية :بكتريا عصوية سالبة لملون كرام،و مستعمراتها دائرية ،ومنتظمة الحواف،ومتألقة المظهر ،وسميكة بعض الشيء ،ولزجة الى قليلة اللزوجة،و بكتريا مخمرة للاكتوز وتعود هذه العزلة الى عائلة *Enterobacteriaceae*.

أظهرت نتائج اختبار الحساسية ضد المضادات الحياتية بأن *E. coli* كانت حساسة لجميع المضادات الحياتية المستعملة عدا الأرثرومايسين ((Erythromycin (E) ،و كلوكساسلين ((Cloxacellin (CX)،و الرفامبين ((Rifampin (RA)، و السيفالوثين ((Cephalothin(KF) ،و الامبسلين ((Ampicillin (AM) ،و البنسلين ((Penicillin (P) . و قد بينت تجربة أختبار حساسية *E. coli* ضد المضادات الحياتية في أوساط زرعية حاوية على تراكيز مختلفة من دواء الأومبرازولomeprazole، الدواء المثبط لضخ البروتون، تعزيز المقاومة من خلال قلة حساسية *E. coli* ألى المضادات التي كانت حساسة لها عكسيا مع تركيز الدواء.

أوضحت النتائج ان الأومبرازول omeprazole قد غيّرَ إمكانيةَ الغشاءِ الخلوي في *E. coli* من خلال إزالةِ إستقطاب غشاءِ الخليةِ نتيجة لتثبط عمل آليةِ ضخِّ البروتونَ، مما عرقل نفوذية المضادات الحياتية الى داخل الخلية البكترية.