To cite this article: Gumus D, Kalayci Yüksek F, Uz G, Bayraktar A, Koseoglu P, Ofluoglu Y, Daylan B, Kucuker M. The effects of insulin and glucose on growth and expression of mar regulon in E. coli Turk J Clin Lab 2019; 10: 209-216.

Original Article

The effects of insulin and glucose on growth and expression of *mar* regulon in *E. coli*

İnsulin ve glukozun E. coli'de üreme ve mar regulonunun ekspresyonu üzerine etkisi

Defne GUMUS*[1], Fatma KALAYCİ YUKSEK[1], Gulsen UZ[2], Asli BAYRAKTAR[2], Pinar KOSEOGLU[2], Yagmur OFLUOGLU[2], Benay DAYLAN[2], Mine KUCUKER[1]

[1]Istanbul Yeni yuzyil University, Faculty of Medicine, Department of Medical Microbiology, Istanbul/TURKEY
[2]Istanbul Yeni yuzyil University, Faculty of Arts and Science, Department of Molecular Biology and Genetics, Istanbul/TURKEY

Abstract

Aim: Host factors are known to modulate virulence, antibiotic susceptibility and growth of bacteria. In the present study we aimed to investigate the effect of human insulin and glucose on growth and expression of mar regulon (*marA, marR, ompF, acrA, acrB and tolC*) of *E. coli* SPC105.

Material and Methods: *E. coli* SPC105 was grown in tryptic soy broth (TSB-control) and TSB containing 20 µU/mL insulin, 200 µU/mL insulin, 0.1% glucose and 200 µU/mL insulin + 0.1% glucose. Growths were determined via turbidimetric method. Quantitative polymerase chain reaction (qPCR) was used to determine the gene expression levels. Statistical analysis were performed via Tukey's post hoc-test.

Results: According to absorbance values, it has been shown that 0.1% glucose and 200 µU/mL insulin + 0.1% glucose have led a significant decrease on growth. Expression of *acrA* gene was shown to be decreased in the presence of 200µIU insulin. Expressions of *marR* and *marA* were also decreased in 200µU insulin, 0.1 % glucose+200µU insulin and 0.1% glucose added TSB. Decreasing of *marA*, *marR* and *acrA* expressions were shown to be statistically significant ($p<0.05$).

Conclusion: The results of the present study has shown once more that, host factors may influence the growth of a bacterium as well as gene expressions associated with antibiotic susceptibilities.

Keywords: glucose; insulin; mar operon; gene expression; growth; *E. coli*

Corresponding author*: Defne Gümüş, Istanbul Yeniyuzyil University, Faculty of Medicine, Department of Medical Microbiology, Istanbul/TURKEY
E-mail: defne.gumus@yeniyuzyil.edu.tr
ORCID: 0000-0003-4070-6924
Recevied: 12.09.2018 accepted: 20.10.2018
Doi: 10.18663/tjcl.459417

Volume 10 Number 2 p: 209-216

Öz

Amaç: Konak faktörlerinin bakterilerin virulans, antibiyotik duyarlılığı ve üreme gibi özelliklerini etkilediği bilinmektedir. Bu çalışmada insan insulin ve glukozun *E. coli* SPC105 suşunun üremesi ve marregulonunun (*marA, marR, ompF, acrA, acrB vetolC* genlerini içeren) ekspresyonu üzerine etkisinin araştırılması hedeflenmiştir.

Gereç ve Yöntemler: *E. coli* SPC105 triptik soy buyyonda (TSB-kontrol) ve 20 µU/mLinsulin, 200 µU/mLinsulin, %0,1 glukoz ve 200 µU/mLinsulin + %0,1 glukoz içeren TSB'de üretilmiştir. Üremeler türbidimetrik yöntem ile belirlenmiştir. Gen ekspresyonu düzeyleri kantitatifpolimeraz zincir reaksiyonu (qPCR) ile araştırılmıştır. İstatistiksel analizlerde Tukey'in post hoc-testi kullanılmıştır.

Bulgular: Absorbans sonuçlarına göre %0,1 glukoz ve 200 µU/mLinsulin + %0,1 glukoz içeren besiyerleri üremeyi anlamlı düzeyde düşürmüştür. *acrA* geninin ekspresyon seviyesinin 200µU/mL insülin içeren besiyerinde düştüğü görülmüştür. *marR* ve *marA*'nın ekspresyonları da benzer şekilde 200 µU/mLinsulin, %0,1 glukoz ve 200 µU/mLinsulin + %0,1 glukoz içeren TSB'de azalmıştır. *marA*, *marR*, *acrA* ekspresyonlarındaki azalış istatistiksel olarak anlamlı bulunmuştur ($p<0.05$).

Sonuç: Bu çalışmanın sonuçları bir kez daha göstermiştir ki, konak faktörleri bir bakterinin üremesi üzerine olduğu kadar antibiyotik direnci ile ilişkili farklı genlerinde ekspresyonu üzerinde etkili olabilmektedir.

Anahtar kelimeler: glukoz; insulin; mar operon; gen ekspresyonu; üreme; *E. coli*

Introduction

The multiple antibiotic resistance (mar) locus first described in the early 1980's in *E. coli* and it is located on the chromosome of all Enterobacteriaceae members. This locus is responsible for the susceptibility of bacterium to all kind of antimicrobial agents unless a plasmid or transposon carrying resistance genes acquired [1, 2]. Mar locus consist of two operons (*marC* and marRAB) and an operator *marO*. The *marC* gene specifies a inner membrane protein of unknown function and is conserved in a number of genera whereas *marR* encodes for a small protein also of unknown function found only in *E. coli* [2]. The *marR* encodes for multiple antibiotic resistance repressor whereas *marA* acts as multiple antibiotic resistance activator. When marRis active, marRAB expression is repressed; when it is inactivated by small molecules or mutated, *marA* transcription occurs,which leads to increase resistance to unrelated chemicals such as quinolones, tetracycline, β-lactams, organic solvents, oxidative stress agents and household disinfectants [1-8]. The product of *marA* also alters the expression of specific genes within the mar regulon. The mar regulon comprised of some genes as follows:*acrA, acrB, marA, marR, ompF* and *tolC* genes [1, 9].

During infection, bacteria must withstand stress conditions induced by diverse environmental factors in their host, such as hormones and many other chemicals (sugars, vitamins, ions, pH, bile salts, and microbial metabolites such as indole) which directly affect microbial survival and other biologically important processes, such as growth and gene expression [10]. In several studies, it has been shown that mammalian hormones such asEpinephrine (E), norepinephrine (NE), dopamine, dopa, estrogen, progesterone, serotonin, testosterone, and insulin were shown to affect bacterial growth, gene expression, pathogenicity and antibiotic susceptibility in numerous reports [11-18]. According to previous studies, the induction of marA has been shown phenotypically within the presence of various herbs, spices, foods and drinks in addition to antimicrobial compounds [19].

In the present study we aimed to investigate the effects of insulin and glucose, as host factors, on growth and expressions of *acrA, acrB, marA, marR, ompF* and *tolC* genes associated with mar regulon in *E. coli* SPC105.

Materials and Methods

Strain

E. coli SPC105 strain containing a chromosomal PmarII::lacZ fusion at the λ attachment site was used. This strain kindly provided by Dr. Stuart Levy and Dr. Valérie Duval from Tufts University School of Medicine, USA. Organism was kept at -80°C for qPCR analysis.

Media and solutions

Tryptic soy broth (TSB) with and without insulin-r (recombinant insulin) (20 µU/ml and 200 µU/ml) and/or glucose (0.1% v/v) were used in the experiments. Insulin provides the storage and cellular uptake of glucose, regulates the carbohydrate and lipid metabolism. Furthermore, insulin is known to highly conserved protein among various taxonomic kingdoms and its blood level is approximate 10µIU/mL in a healthy individual. The reabsorption only occurs for the 98% of the total insulin while the 2% of it excreted in urine [20-22]. Two insulin concentrations used in the present study have been determined as 2 and 20 times more than the normal blood levels. The glucose concentration was determined according to its physiological blood level concentration (100 mg/dL)[11, 20-22].

Effects of insulin and glucose on growth

For the detection of growth alterations turbidimetric method was used. Overnight culture of *E. coli* SPC105 strain was inoculated into TSB medium alone (as control) or supplemented with 20 µU/mL insulin, 200 µU/mL insulin, 0.1% glucose and 200 µU/mL insulin + 0.1% glucose to an initial turbidity of ~ 107CFU/mL. Microorganisms were incubated at 37°C. Growth in different experimental cultures was determined by the measurement of changes in absorbance 600nm on 4th, 6th and 24th hours of incubation.

To relate the turbidimetric measurement to direct counting, a standard curve has been prepared with inoculation of bacteria in TSB. Absorbance values were transformed to CFU/mL for all different conditions.

Effects of insulin and glucose on gene expressions

Gene expressions were determined according to transcriptional level of mRNAs.

Total RNA extraction and cDNA synthesis

E. coli SPC105 was grown in TSB (control), TSB + 20 µU/mL insulin, TSB + 200 µU/mL insulin, TSB + 200 µU/mL insulin + %0,1 glucose and TSB + %0,1 glucose for 16-24 hours at 37^0C by shaking 200 rpm. Bacterial cells of overnight culture were used in total RNA extraction. Total RNAs were obtained from broth cultures of 1.5 mL by using total RNA isolation kit (Hibrigen, Turkey). The manufacturer's recommendations were followed in total RNA extraction. DNAseI treatment was carried out by using DNaseI kit according to the manufacturers recommendations (Quanta, Germany). Qualitative analysis of RNAs was carried via 1% agarose gel electrophoresis and bands were visualized via gel documentation system (Maestrogen, USA). Qualitative analysis of total RNAs was maintained by using spectrophotometer (Shimadzu, Japan).

1 µg total amount of RNAs were used in cDNA synthesis. cDNA synthesis was maintained in a reaction volume of 20 µL using a two stepc DNA synthesis commercial kit (Vivantis, Malaysia). The reaction mix was formed according to manufacturer's provided protocol. cDNA mixture was diluted with 1:4 dilution factor and used in qPCR analysis.

Gene Expression Assays

qPCR (quantitative real time PCR) analysis was carried out based on Eva Green (Bio-Rad, France) dye with 500/530 nm excitation/emission values. The endogenous gene was 16S rRNA gene. *marA, marR, ompF, acrA, acrB* and *tolC* genes were used as target genes. Experiments were carried out at least three times. Standard curves were formed by four logs dilution series and PCR efficiency values were recorded for each gene separately. Melting curve analysis was carried out in order to analyze the accuracy of experiments. Cp values were obtained from Light Cycler 480 II qPCR system's software (Roche, Switzerland). Normalization (2-ΔΔCT) values were calculated according to formula developed by Pfaffl (2001). PCRs were conducted in a reaction volume of 24 µL including 1X Eva Green mix, 2 pmol of each primer (Table 1), 2 µL of cDNA and nuclease free water. Cycling conditions were followed as 95°C for 5 minutes, 45 cycles of 95°C for 15 seconds, 58°C for 15 seconds, 72°C for 20 seconds, and final extension at 72°C for 10 minutes [23]. The results were expressed as the mean of three independent experiments. Variance analysis via one-way ANOVA test was carried out using GraphPad Prism 5.0 software (San Diego California, USA).

Qualitative analysis of gene expression was carried out by using two step conventional reverse transcription PCR (RT-PCR). PCR cycling conditions were performed at 94°C for 5 minutes for predenaturation, 40 cycles at 94°C for 30 seconds, 58°C for 30 seconds, 72°C for 45 seconds, and at 72°C for 5 minutes for final extension. PCR bands were analyzed via 1.5% agarose gel electrophoresis and gel imagination system Gel Pro Analyzer 3.2 software.

Volume 10 Number 2 p: 209-216

Table1. *Primers used in the gene expression analysis

Gene	Sequence (5'-3')	Band size in bp
16S rRNA-f 16S rRNA-r	CCA GGA TTT GAT YMT GGC GAA GGA GGT GWT CCA DCC	532
marA-f marA-r	TTA GGC CAA TAC ATC CGC AG AAG GTT CGG GTC AGA GTT TG	128
marR-f marR-r	TGT AAA GGC TGG GTG GAA AG GTT AAT TCT TGG TGC AGG TCC	134
ompF-f ompF-r	GGTGTTGGCGGTTCTATCAG TTCTTGCAGGTTGGTACGG	87
acrA-f acrA-r	CAT TGG TAC AGA ACG GTC AGG GTT CTC TTG TTT CAG CGT GC	140
acrB-f acrB-r	TTC CAT CTT CGC CAG TTC AG TCA TCG CAG AGT TTA ACG GC	113
tolC-f tolC-r	CGG GAT TTC TGA CAC CTC TTA TAG ACC TGC GAG TTA ACC ATT CC	144

*Primer sequences were prepared for present study (https://eu.idtdna.com/scitools/Applications/RealTimePCR/)

Statistical analysis

Each assay was performed in duplicate, and the results were expressed as the mean of two independent experiments. Statistical analysis of growth alterations were performed by using a two-way ANOVA Bonferronipost test. Significant differences in gene expressions were analysed by using Tukey's post hoc-test.

Results

Effects of insulin and glucose on growth

The growth of E. coli SPC105 in TSB supplemented with 200µU insulin+0.1% glucose and 0.1% glucosewere found decreased only in the 24 hour period. The differences between supplemented TSBs mentioned above andTSB without insulin and/or glucose were found to be statistically significant ($p<0.001$). But 20µU insulin and 200µU insulin were not affected the growth of bacterium.

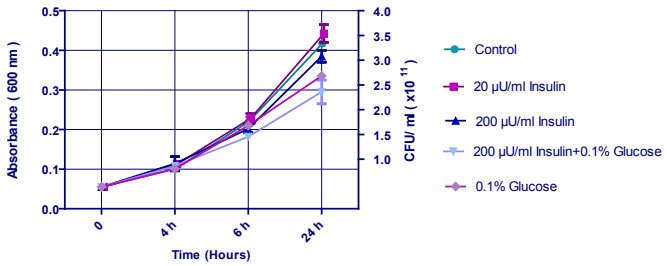

Figure 1. Effects of different insulin concentration and glucose on growth of *E. coli* SPC105.

E. coli was grown in TSB medium at 37 °C for 24 hours in the presence of 20 µU/mL insulin, 200 µU/mL insulin, 0.1% glucose and 200 µU/mL insulin + 0.1% glucose. Data shown mean absorbance ± standard deviation.

Effects of insulin and glucose on gene expressions Real Time PCR assays

High quality of total RNAs with Δ260/280 ~1.9-2.0 were obtained via commercial total RNA isolation kit. Converted RNAs were subjected to qPCR and RT-PCR assays. Melting scores were detected as 90-100%. Mean cross point values Cp together with their standard deviation related to each gene of all conditions were given in Table 2. Minimum and maximum Cp values were 11.87±0.59 and 28.84±0.95, respectively.

Table 2. Crossing point values belonging to each gene related to each condition.

Genes	Cpvalues				
	Control	20 µU/mL insulin	200 µU/mL insulin	0.1% glucose	200 µU/mL insulin + 0.1% glucose
16s rRNA Mean	13.29 ± 0.8	12.14±0.2	12.46±0.6	11.87±0.05	12,42±1.19
acrA Mean	27.47±0.78	26.70±0.15	27.25±0.4	26.65±0.25	26.90±1.19
acrB Mean	28.84±0.96	27.93±0.07	28.44±0.56	27.85±0.03	27.99±1.28
marA Mean	24.13±1.18	23.30±0.22	24.16±0.34	23.73±0.16	24.42±0.98
marR Mean	25.82±0.96	24.99±0.45	25.70±0.05	24.90±0.26	25.93±0.67
omp-f Mean	27.10±0.87	26.20±0.15	26.90±0.34	26.63±0.29	26.83±1.55
tol-C Mean	27.70±0.16	27.24±0.09	27.55±0.65	27.05±0.19	27.25±1.2

The lowest PCR efficiency value (1.87±0.21) was recorded in endogenous gene. Fold changes in gene expression were shown in graphics of six target genes for four experiment groups and the control group. (Figure 2). Expresions of *marA* and *marR* were found to be down regulated significantly in the presence of 200 µU/mL insulin, 0.1% glucose and 200 µU/mL insulin + 0.1% glucose. 200 µU/mL insulin has also shown to decrease the expression of *acrA*. Fold changes in gene expression were ranged from 0.46±0.16 to 1.2±0.26 (Table 3).

Table 3. Fold changes in gene expression.

Target Genes	*Fold Changes				
	Control	20 µU/mL insulin	200 µU/mL insulin	0.1% glucose	200 µU/mL insulin + 0.1% glucose
acrA	1.0±0.0	0.76±0.12	0.56±0.03**	0.63±0.06	1.03±0.14
acrB	1.0±0.0	1.0±0.3	0.76±0.2	0.83±0.2	1.2±0.26
marA	1.0±0.0	1.1±0.34	0.53±0.12***	0.53±0.12**	0.5±0.1***
marR	1.0±0.0	0.73±0.12	0.5±0.05***	0.63±0.08**	0.5±0.05***
ompF	1.0±0.0	0.86±1.16	0.6±0.05	0.93±0.43	0.86±0.26
tolC	1.0±0.0	0.56±0.17	0.53±0.13	0.46±0.16	0.66±0.26

*Gene expression levels (fold chances) were calculated according to 2 -ΔΔCT values.
**: p< 0.05; ***: p<0.01

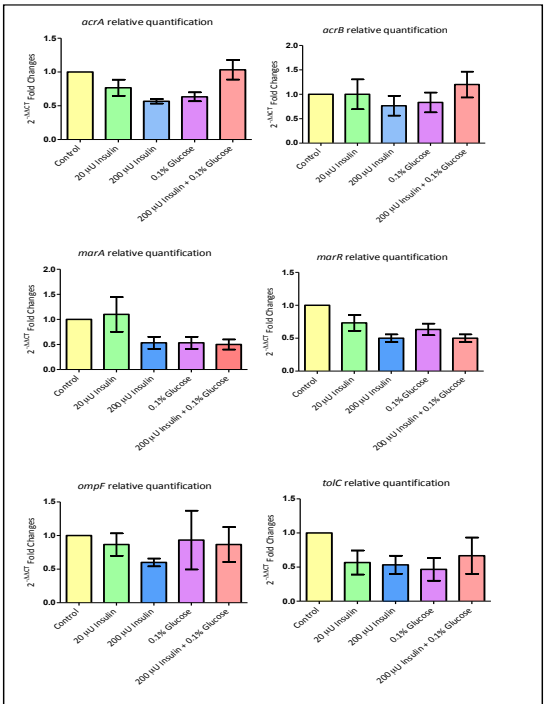

Figure 2. Fold changes in gene expression. Gene expressions were determined in mRNA transcriptional level.

Discussion

In Mar phenotypes (Mar mutants) transcription of marRABoperon increases either as a result of mutations in regulatory region *marO* or in *marR* [5]or in the presence of different inducer chemicals (antimicrobials, glossaries, disinfectants, hormones, nutrition etc.) which are able to bind to *marO* [3,5, 24, 25]. Transcription ofmarRAB induces the expression of *mar A* controlling acrAB - tolC and omp Fwhich play roles in multidrug efflux pumps and porin production [25-28]. In wild type strains acrAB-tolC (efflux system) is expressed at low levels, but when up regulated by *marA*, it causes high level resistance to broad-range of antibiotics, lipophilic drugs, detergents, organic solvents, ethidium bromide, crystal violet, bile salts, beta-lactams, novobiocin, erytromicin, fusidic acid, tetracycline, chloramphenicol [19, 29, 30]. Furthermore *marA* provides inhibition of translation on ompF mRNA which causes decreasing the entry of various antibiotics which defined as non-specific solute transporter [31, 32]. It is know that multi-resistant *E. coli* strains have lower levels of OmpF [33].

Various host factors [epinephrine (E), norepinephrine (NE), dopamine, dopa, estrogen, progesterone, serotonin, testosterone, insulin and different nutritious compounds] are shown to affect many physiological processes in a bacterium as a result of inter-kingdom communication [11, 13-21, 34, 35]. Previous studies suggested that, insulin-r may function as an inter-kingdom QS compound which regulates growth, biofilm formation and chemotactic responses in *E. coli* [10, 11, 36].

Studies investigated the expressions of antimicrobial resistance genes were mostly focused on interactions of yeast and steroid hormones [37, 38]. Steroids are known to affect the expression levels of multidrug resistance genes (CDR1 gene) in Candida species. According to the study of Michelli et al., [37] expressions of CDR1 and CDR2 genes (related to azole resistance) were up regulated in the presence of estradiol while it had no effect on growth of Candida. Similar to these results, Banarjee et al. [38] reported that, progesterone enhanced the expression of multidrug resistance genes. Plotkin et al. [36]showed that, dehydroepiandesterone increased the vancomycin resistance in *Staphylococcus aureus* not only phenotypically but also in molecular level. In addition glucose as another host factorhas been shown to be altered antibiotic susceptibility of different types of bacteria. Peng et al (2015) reported that exogenous glucose altered susceptibility of multi drug resistant *Edwardsiella tarda* to kanamycin. Researchers suggested that exogenous glucose affects TCA cycle which increases drug intake [39]. Allison

et al (2011) reported that addition of glucose, mannitol and fructose affect the metabolic pathways of *E.coli* and *S.aureus*, which leads the eradication of biofilm formations [40].

As we all know, the growth of bacteria such as *Pseudomonas aeruginosa*, *Bacteroides melaninogenicus, Campylobacter rectus, E. coli, Staphylococcus aureus, Enterococcus faecalis,* and Burkholderia pseudomailei was altered in the presence of catecholamine, gender hormones, and insulin [11, 16, 41-43]. According to the study of Woods et al. the growth of B. pseudomailei in minimal medium containing human insulin-r was significantly lower when compared to control cultures [44]. Plotkin and Viselli showed that, the growth kinetics of *E. coli*, *P. aeruginosa, S. aureus,* and *E. faecalis* were affected by the addition of insulin (2, 20, 200, and 400 µU/mL insulin were used), and glucose (1%, 5%) in Mueller-Hinton Broth [11]. Bakholdina et al (2004) shown that, addition of 0.5% glucose leads to inhibition of Yersinia *pseudotuberculosis growth* [45]. Gümüş et al (2017) reported that no change was detected on growth ofUropathogenic *E. coli* strain (C7) in the presence of insulin and/or glucose [10]; but in the present study the growth of SPC105 *E. coli* strain was found to be affected in the same conditions. These results supported that host factors could modulate the growth of bacteria in a strain dependent manner.

To our knowledge this is the first paper focusing on interactions of expressions of antibiotic resistance genes with human recombinant insulin and glucose. Mar regulon as an intrinsic source for antibiotic resistance known to be affected in the presence of different chemicals, glossaries and antibiotics [19]. Transcription of *marRAB* operon is normally repressed unless inducer chemicals are present in the environment of bacteria such as tetracycline, chloramphenicol, sodium salicylate and other unrelated compounds. In the present study, we aimed to investigate the possible effects of human insulin and glucose on mar regulon with Q-PCR.

According to our results, among the sixinvestigated genes (*acrA, acrB, tolC, ompF, marA, marR*) only *acrA*, *marR* and *marA* were shown to be down regulated in the presence of insulin and/or glucose when compared to control media (TSB). However, the down regulation level of *marR* (in the presence of 200µU insulin, 200µU insulin+0.1% glucose and 0.1%glucose) did not lead to induction of *marA* as it is always expected. Therefore we considered that, the expression of *marA* and *marR* were affected independently. As we all know, the activation of efflux proteins (acrAB-tolC) are regulated by expression of *marA*. However in the present study, expression of *acrA* was also down regulated independently from *marA*.

As the main environment for a microorganism, host factors (hormones, antibiotics, nutrition) act as signal molecules during long term exposure such as infection. Consistent with previous studies our results prove that, these host factors (insulin and glucose for instance) affect gene expression (both virulence and antibiotic resistance) and growthof bacteria in vitro [10, 11, 36]. Today it is well known that, the regulation of drug resistance and virulence mechanisms are tightly connected to each other [46]. In accordance with the previous studies our results show that, insulin and glucose can affect the growth and expression levels of different genes related to virulence but also antibiotic resistance [10-12, 36].

Declaration of conflict of interest

The authors received no financial support for the research and/or authorship of this article. There is no conflict of interest.

Acknowledgements

We would like to thank Dr. Emre Yörük from İstanbul Yeni Yüzyıl University, Department of Molecular Biology and Genetics for his technical support. This research was supported by TUBITAK (The Scientific and Technological Research Council of Turkey) (Project No: 2209-a / 2016).

References

1. Alekshun MN, Levy SB. The mar regulon: multiple resistance to antibiotics and other toxic chemicals. Trends Microbiol 1999; 7: 410-13.
2. Alekshun MN,Levy SB.The Escherichia coli mar Locus—Antibiotic Resistance and More. ASM News 2004; 70: 451-56.
3. George AM, Levy SB. Amplifiable resistance to tetracycline, chloramphenicol, and other antibiotics in Escherichia coli: involvement of a non-plasmid-determined efflux of tetracycline. J Bacteriol1983; 155: 531-40.
4. Piddock LJ. Multidrug-resistance efflux pumps - not just for resistance. Nat Rev Microbiol 2006; 4: 629-36.
5. Cohen SP, LevySB, FouldsJ, RosnerJL. Salicylate induction of antibiotic resistance in Escherichia coli: activation of themaroperon and a mar-independent pathway. J Bacteriol 1993; 175: 7856-62.
6. Oethingera M, Kernc WV, Goldmana JD, Levy SB. Association of organic solvent tolerance and fluoroquinolone resistance in clinical isolates of Escherichia coli. J Antimicrob Chemother1998; 41: 111-14.
7. Ferrari RG, Galiana A, Cremades R et al. Expression of the mar A, sox S, acr B and ram A genes relatedto the Acr AB/TolC efflux pump in Salmonella enterica strains with and without quinolone resistance-determining regions gyr A gene mutations. The Brazilian Journal of Infectious Diseases 2013; 17: 125-30.

8. Maneewannakul K, Levy SB. Identification for mar mutants among quinolone-resistant clinical isolates of Escherichia coli. Antimicrob Agents Chemother 1996; 40:1695-98.
9. Alekshun MN, Levy SB.Regulation of chromosomally mediated multiple antibiotic resistance: the mar regulon. Antimicrob Agents Chemother1997; 41: 2067-75.
10. Gumus D, Yoruk E, Kalayci-Yuksek F, Uz G, Topal-Sarikaya A, Ang-Kucuker M.The Effects of Insulin and Glucose on Different Characteristics of a UPEC: Alterations in Growth Rate and Expression Levels of someVirulence Genes. Clin Lab 2017; 63:1589-97.
11. Plotkin BJ, Viselli SM. Effect of insulin on microbial growth. Curr Microbiol 2000; 41: 60-64.
12. Gönüllü N, Küçükbasmacı Ö, Büyükbaba-Boral Ö, Anğ-Küçüker M. The influence of glucose added urine on the in vitro antimicrobial activity of various antibiotics. Indian J Med Res 2008;128: 663-65.
13. Lyte M, Ernst S. Catecholamine induced growth of gram negative bacteria. Life Sci 1992; 50: 203-12.
14. Freestone PP, Haigh RD, Lyte M. Blockade of catecholamine-induced growth by adrenergic and dopaminergic receptor antagonists in Escherichia coli O157: H7, Salmonella enterica and Yersinia enterocolitica. BMC Microbiol 2007; 7: 8.
15. Merkel SM, Alexander S, Zufall E, Oliver JD, Huet-Hudson YM. Essential Role for Estrogen in Protection against Vibrio vulnificus-induced Endotoxic Shock. Infect Immun2001; 69: 6119-22.
16. Yokoyama M, Hinode D, Masuda K, Yoshioka M, Grenier D. Effect of female sex hormones on Campylobacter rectus and human gingival fibroblasts. Oral MicrobiolImmunol 2005; 20: 239-43.
17. Sandrini S, Alghofaili F, Freestone P, Yesilkaya H. Host stress hormone norepinephrine stimulates pneumococcal growth, biofilm formation and virulence gene expression. BMC Microbiology 2014; 14: 180.
18. Clark DT, Soory M. The influence of cholesterol, progesterone, 4-androstenedione and testosterone on the growth of Treponemadenticola ATCC 33520 in batch cultures. Anaerobe 2006; 12: 267-73.
19. Rickard AH, Lindsay S, Lockwood GB, Gilbert P. Induction of the mar operon by miscellaneous groceries. Journal of Applied Microbiology 2004; 97:1063–68.
20. LeRoith D, Shiloach J, Roth J, Lesniak MA. Insulin or a closely related molecule is native to Escherichia coli. J Biol Chem 1981; 256: 6533-36.
21. LeRoith D, Pickens W, Vinik AI, Shiloach J. Bacillus subtilis contains multiple forms of somatostatin like material. Biochem Biophys Res Commun1985; 127: 713-19.
22. Aun F, Soeldner JS, Meguid MM, Stolf NA. Urinary insulin levels in health and disease-a concise review. Postgrad Med J 1975; 51: 622-26.
23. Pfaffl MW. A new mathematical model for relative quantification in real-Time RT-PCR. Nucleic Acids Research 2001; 29: 2003-2007.
24. Randall LP, Woodward MJ. Multiple antibiotic resistance (mar) locus in Salmonella entericaserovartyphimurium DT104. Appl Environ Microbiol 2001; 67: 1190-97.
25. Barbosa TM, Levy SB. Differential expression of over 60 chromosomal genes in Escherichia coli by constitutive expression of MarA. J Bacteriol 2000; 182: 3467-74.
26. Blair JM, Piddock LJ. Structure, function and inhibition of RND efflux pumps in Gram-negative bacteria: an update. Curr Opin Microbiol 2009; 12: 512-19.
27. White DG, Maneewannakul K, Von Hofe E, Zillman M, Eisenberg W, Field AK, Levy SB. Inhibition of the multiple antibiotic resistance (mar) operon in Escherichia coli by antisense DNA analogs. Antimicrob Agents Chemother 1997; 41: 2699-704.
28. Zgurskaya HI, Krishnamoorthy G, Ntreh A, Lu S. Mechanism and Function of the Outer Membrane Channel TolC in Multidrug Resistance and Physiology of Enterobacteria. Front Microbiol 2011; 2: 189.
29. Chollet R, Chevalier J, Bollet C, Pages JM,Davin-Regli A. RamA Is an Alternate Activator of the Multidrug Resistance Cascade in Enterobacteraerogenes. Antimicrob Agents Chemother 2004; 48: 2518–23.
30. White DG, Maneewannakul K, Von Hofe E, Zıllman M, Eisenberg W, Field AK, Levy SB. Inhibition of the Multiple Antibiotic Resistance (mar) Operon in Escherichia coli by Antisense DNA Analogs. Antimicrob Agents Chemother 1997; 41: 2699–704.
31. Cohen SP, McMurry LM, Levy SB.marA locus causes decreased expression of OmpFporin in multiple-antibiotic-resistant (Mar) mutants of Escherichia coli. J Bacteriol 1988; 170: 5416-22.
32. Coyer J, Andersen J, Forst SA, Inouye M, Delihas N.micF RNA in ompB mutants of Escherichia coli: different pathways regulate micF RNA levels in response to osmolarity and temperature change. Bacteriol1990; 172: 4143-50.
33. Agafitei O, Kim EJ, Maguire T, JaredS. The Role of Escherichia coli Porins OmpC and OmpF in Antibiotic Cross Resistance Induced by Sub-inhibitory Concentrations of Kanamycin.Journal of Experimental Microbiology and Immunology (JEMI) 2010; 14: 34-39.

34. Freestone PP, Sandrini S: Mechanisims by which cathecholamines ınduce growth in Gram negative and Gram positive human pathogens. In:Microbial Endocrinology: Inter kingdom Signaling in Infectious Disease and Health, Lyte M. Freestone PP (eds). 1sted. New York: Springer; 2010: 53-68.

35. Lyte M. Bacteria and hormones: Why the science of microbial endocrinology matters to disease and well-being. In: Bacterial species as partners and pathogens, Heidt PJ, Midtvedt T, Rusch V, Versalovic J (eds). Herborn-Dill: Old Herborn University Foundation; 2012: 17-29.

36. Plotkin BJ, Konakieva MI. Attenuation of antimicrobial activity by the human steroid hormones. Steroids 2017; 128: 120–127.

37. deMicheli M, Jacques B, Schueller C, Sanglard DA. Common drug-responsive element mediates the upregulation of the Candida albicans ABC transporters CDR1 and CDR2, two genes involved in antifungal drug resistance. Molecular Microbiology 2002; 43: 1197–214.

38. Banerjee D, Martin N, Nandi S, Shukla S, Dominguez A, Mukhopadhyay G, Prasad R. A genome-wide steroid response study of the major human fungal pathogen Candida albicans. Mycopathologia 2007; 164: 1–17.

39. Peng B, Ma YM, Zhang JY, Li H. Metabolome strategy against Edwardsiellatarda infection through glucose-enhanced metabolic modulation in tilapias. Fish Shellfish Immunol 2015; 45: 869-76.

40. Allison KR, Brynildsen MP, Collins JJ. Metabolite-enabled eradication of bacterial persisters by aminoglycosides. Nature 2011; 473: 216-21.

41. Li W, Lyte M, Freestone PP, Ajmal A, Colmer-Hamood JA, Hamood AN. Norepinephrine represses the expression of toxA and the siderophore genes in Pseudomonas aeruginosa. FEMS Microbiol Lett 2009; 299: 100-109

42. Kornman KS, Loesche WJ. Effects of estradiol and progesterone on Bacteroidesmelaninogenicus and Bacteroidesgingivalis. Infect Immun1982; 35: 256-63.

43. Intarak N, Muangsombut V, Vattanaviboon P, Stevens MP, Korbsrisate S. Growth, motility and resistance to oxidative stress of the melioidosis pathogen Burkholderiapseudomallei are enhanced by epinephrine. Pathog Dis 2014; 72: 24-31.

44. Woods DE, Jones AL, Hill PJ. Interaction of insulin with Pseudomonas pseudomallei. Infect Immun1993; 61: 4045-50.

45. Bakholdina SI, Sanina NM, Krasikova IN, Popova OB, Solov'eva TF. The impact of abiotic factors (temperature and glucose) on physicochemical properties of lipids from Yersinia pseudotuberculosis.Biochimie 2004; 86: 875-81.

46. Schroeder M., Brooks BD., Brooks AE. The Complex Relationship between Virulence and Antibiotic Resistance. Genes2017; 8: 1-23.