**ORIGINAL ARTICLE**

# A Comparison of Human Gut Microbiota and Analysis of Antimicrobial Resistance of *E. coli* Isolates in Saudi Adults Undergoing Bariatric Surgery

***Kawther Aabed[1], Nadine Moubayed[2], Abrar Motlak[3], Al-Anoud Al-Rasheed[4], Ameerah Al-Otaibi[5] Aisha Al-Asmri[6], Hanadi Abu-Dusha[7]***

**ABSTRACT**

***Background and Objective*:** Human gut harbors a diverse community of more than 100 trillion microbial cells that play an important role in human metabolism, physiology, immune function, and nutrition. Similarly, disruptions to the composition of this population can be linked with gastrointestinal conditions, such as obesity and inflammatory bowel disease. The aim of the present study was to compare the microbial community characteristics among obese, normal-weight and post-gastric bariatric surgery patients.

***Methods*:** Fifteen healthy adults, who were classified into (1) normal-weight (*n*=5) (2) obese (*n*=5) according to their Body mass index (BMI) and (3) post-bariatric surgery (*n*=5) groups. Gut microbiota from fecal samples were profiled by the streak plate method and theconstituent populationswere identified by biochemical analysis (Vitek2). Finally, specific bacterial strains were identified via molecular techniques, such as Polymerase chain reaction (PCR). Moreover, the antimicrobial sensitivity of *E. coli* strains isolated from healthy adults was evaluated to determine the pathogenic strains.

***Results:*** The findings revealed that gut microbiota diversity increased following bariatric surgery, whereby84.6% of increased bacteria belonged to Proteobacteria, especially Escherichia *coli* (member of Gammaproteobacteria). The main bacterial groups in individuals that underwent bariatric surgery were Gemella *morbillorum,* Citrobacter *freundii,* Serratia *odorifera,* Proteus *mirabilis,* Enterococcus *gallinarum,* Klebsiella *pneumonia,* Enterobacter *cloacae* complex, Pseudomonas *aeruginosa* and Escherichia *coli.* The main bacterial groups in the normal-weight group were Citrobacter *freundii,* Serratia *marcescens,* Proteus *mirabilis* and Escherichia *coli*. Finally, in obese individuals, Enterobacter *cloacae* complex, Klebsiella *pneumonia,* Enterococcus *gallinarum* and Escherichia *coli* were the main bacterial groups.

***Conclusion:*** These results indicate that inducing changes in the gut microbiota may be a relevant therapeutic approach for obesity and other metabolic defects.

**KEYWORDS:** Human, Gut microbiota, Obesity, Normal-weight, Bariatric surgery, Adults.

***How to Cite This:***

*Aabed K, Moubayed N, Motlak A, Al-Rasheed AA, Al-Otaibi A, Al-Asmri A, et al. A comparison of human gut microbiota and analysis of antimicrobial resistance of E. coli isolates in Saudi adults undergoing bariatric surgery. Biomedica. 2020; 36 (2): 118-25.*

1 Kawther Aabed
2 Nadine Moubayed
3 Abrar Motlak
4 Al-Anoud Al-Rasheed
5 Ameerah Al-Otaibi
6 Aisha Al-Asmri
7 Hanadi Abu-Dusha

1,3,4,5,6,7: Biology Department, College of Science, Princess Nourah Bint Abdul Rahman University, Riyadh, Saudi Arabia.

2. Biology Department, College of Sciences, King Saud University, Riyadh, Saudi Arabia.

Correspondence to:
Professor Kawther Aabed
Biology Department, College of Science
Princess Nourah Bint Abdul Rahman University
Riyadh, Saudi Arabia.
E-mail:, dr.kaabed@gmail.com

- Received for publication: 07-03-2020
- Revision received: 09-06-2020
- Accepted for publication: 15-06-2020


## INTRODUCTION

Obesity is becoming more prevalent and is now recognized as one of the most significant health issues worldwide. While prior to 1980 only 10% of world population was classified—based on the body mass index (BMI) as obese, according to the Organization for Economic Cooperation and Development (OECD), half the developed countries, 50% of the population being overweight in 2010.[1,2] Obesity is a metabolic syndrome resulting from a prolonged imbalance of energy intake and expenditure.[3] Excess body weight has detrimental impact on the body, as it induces significant changes to regulatory mechanisms and energy metabolism, leading to cardiovascular disease (CVD), hormone-linked cancers, inflammatory bowel disease, and type 2 diabetes.[4] Many environmental and genetic factors, as well as cultural norms, including socio-economic status, nutrient intake, participation in sports and its effects on weight, all of these contribute to the growing prevalence of obesity.[5,6] However, recent reports indicate a potential link between the type and composition of the intestinal microbiota and obesity.[7,8] According to Guo and colleagues 80% of the fecal microbiota identified in healthy adults can be categorized into three dominant phyla: Firmicutes, Bacteroidetes, and Actinobacteria.[9] In general, the Bacteroidetes to Firmicutes ratio is considered to be particularly relevant to the human gut microbiota structure.[7] An increased ratio of Firmicutes to Bacteroidetes in obese individuals relative to their Lean counterparts has been reported, including Clostridium *clusters* in their intestinal microbiota.[7,8] Bariatric surgery is one of the most efficient procedures for treating morbid obesity, as it results in a significant weight loss, as well as marked improvements in metabolism and inflammation. Moreover, weight loss is associated with partial recovery in bacterial components similar to those of lean profiles.[10] Findings yielded by extant studies[7,9] suggest that following bariatric surgery, the gut microbiota is greatly altered with respect to Firmicutes and Bacteroidetes phyla, known to affect human metabolism. However, the effect of intestinal microbes on weight loss following the stomach bypass surgery remains insufficiently explored. Available evidence indicates that, as bariatric surgery decreases the stomach volume, as well as shortens the gut, it significantly diminishes nutrient intake. As a result of malabsorption of nutrients, it affects the intestinal microbiota involved in mediating nutrient metabolism, which in turn affects weight maintenance.[11]

Escherichia *coli* is one of the familiar residents of human digestive tract andis cited in extant literature as the most common reason of nosocomial infections.[12] Within the digestive tract, commensal E. *coli* can transmit its antibiotic-resistant genes to different microorganisms, such as pathogenic organisms, particularly when exposed to antimicrobials.[13] Smith[14] was the first to describe the transfer of antibiotic-resistant genes from E. *coli* strains from the gastrointestinal tract of humans and animals. These results have been proven by many other authors.[15-18] In Saudi Arabia, pharmacies sell antibiotics without prescription, thus contributing to their improper use. Moreover, the available data on antibiotic consumption is unreliable. However, it is widely accepted that improper antimicrobial use practices contribute to the increase in antimicrobial resistance.[19]While these effects differ from one nation to another, the development of antimicrobial resistance is complex and is believed to differ by organism and antimicrobial.[19] These issues were further explored in the present study, the aim of which was to determine the spread of resistant *E. coli* strains in the isolates pertaining to the individuals who were obese, normal-weight, and those who had undergone the bariatric surgery.

To meet these study objectives, traditional methods were adopted to analyze the human gut microbiota in fifteen individuals, comprising of five normal-weight, five obese, and five post-bariatric surgerypatients. Using biochemical tests and/or molecular techniques, the goal was to identify a specific microbial family that may play a significant role in the development of obesity. In addition, this study aims to determine the abundance of these microorganism changes following bariatric surgery. Finally, the antimicrobial sensitivity of E. *coli* strains isolated from humans was examined to determine the pathogenic strains.

## METHODS

### Patient Records

Stool samples were collected from 15 subjects, who were divided into three groups—denoted as normal weight (nw: BMI 20-25 kg/m$^2$), obese (ob: > 35 kg/m$^2$), and post-gastric bypass (gb: 27.7 kg/m$^2$)—with five patients per group. Participants were predominantly female (10 vs. 5 males). Mean (SD) subject age (expressed in years) was similar across the three groups 24.1 (3.8); 36.9 (3.1); and 58.04 (19.5) for nw, ob, and gb, respectively). The mean weight loss after Roux-en-Y gastric bypass (RYGB) was 37.3 (18.04). Post-gastric bypass group stool samples were collected 8 – 15 months after the RYGB. At that stage, weight loss had stopped in two of subjects, whereas the remaining three participants in this groupwere still losing weight. None of the participants lived in the same house, and none had taken any probiotic, prebiotic, or antibiotics agents in the three months earlier the collection of fecal samples.

### Blood Samples

To estimate the lipid profile [total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, triglycerides], 5 ml. of venous blood was taken from subjects (after 12 – 14 h fasting period) under aseptic conditions. Lipid analysis was performed by using the COBAS INTEGRA® 400 plus by Cobas – Roche at Bio House Medical Lab., Riyadh.

### Fecal Sample Collection and Selective Bacterial Culture

Fecal bacteria were obtained from freshly collected human feces under anaerobic conditions. 1:10 fecal were diluted in buffered peptone water solution, comprising of 0.1% (w/v) peptone and 0.85% (w/v) NaCl in distilled water. Next, a volume of 100 µl of this enrichment suspension was placed on different selective agar media, listing Bile Esculin Azide Agar (BEA), MRS, Agar BSM-agar, MacConkey's Agar, and Brain Heart Infusion Broth (BHI) with 1.5% agar (Qingdao Hope Biol-Technology Co, Ltd). These plates were individually incubated at 37°C overnight under different incubation conditions, such as BEA in an aerobic incubator, whereas MRS and BHI were placed in an anaerobic $CO_2$ incubator.

### Isolation and Identification of Strains

All distinct colonies from each plate were identified according to their morphological characteristics. All isolates were purified by streak plate method on Nutrient agar (NA) before being stored at −80°C in a freezing medium[20] until required for analyses. Bacterial colonies were subsequently identified based on their morphological characteristics and microscopic appearance, as well as through a set of biochemical analyses using Vitek2 system (Biome´rieux) using GN test cards.

### PCR Reaction

E. *coli* strains identified using Vitek2, based on the biochemical test findings, were further identified via PCR reaction. For this purpose, bacterial isolates were cultured overnight in a shaking incubator for 18 h at 37˚C in 2 ml Luria Bertani broth (LB). Next, the genomic DNA was extracted using the Invitrogen DNA pure link kit(Invitrogen, Science life, USA) to be amplified further with the use of primers specific for the gene *tuf*TEcol553:5'-TGGGAAGCGAAAATCCTG-3' and Tecol754: 5'CAGTACAGGTAGACTTCTG-3'. The amplification reactions were performed in a total reaction volume of 25 µl using the following program: an initial denaturation 94ºC for 5 min, and a second denaturation at 94ºC for 30s, primers annealing at 58ºC for 1 min, last step (extension) at 72ºC for 30s, and a final extension at 72ºC for 10 minutes. The concentration and purity of the resulting PCR products were then measured using the Genova Nano spectrophotometer (Bibby Scientific Ltd, Italy). Finally, the PCR products were identified by 1.5% agarose gel electrophoresis in parallel to the standard *E. coli* (ATCC 25996).

### Escherichia *coli* Isolates and Antimicrobial Sensitivity Profile

Antimicrobial sensitivity testing was performed on all isolates using the Vitek2 system [Biome´rieux]. The following antimicrobials were examined using AST-N291 test cards: Extended-Spectrum-Beta-Lactamase (ESBL), amoxicillin, ampicillin,

ampicillin/clavulanic acid, ampicillin/sulbactam, piperacillin/tazobactam, cefotetan, cefotaxime, cefoxitin, cefixime, cefuroxime, ceftazidime, ceftriaxone, cefepime, aztreonam, imipenem, meropenem, amikacin, gentamicin, ciprofloxacin, norfloxacin, ofloxacin, doxycycline, minocycline, tigecycline, nitrofurantoin, and trimethoprim/sulfamethoxazole. *E. coli* ATCC 25966 was used as the control strain. The minimum inhibitory concentrations of all *E. coli* strains were determined by agar dilution using the Clinical and Laboratory Standards Institute (CLSI) protocols.

## Ethical Approval

This study was conducted in accordance with the Declaration of Princess Nourah University and was approved by the Institutional Review Board (approval number H-01-R-059). All study participants provided written informed consent.

## STATISTICAL ANALYSIS

Quantitative data were statistically analyzed in terms of mean values and standard deviations (SD). A comparison between different groups in the present study was made using Student's t-test for comparing between variables. A probability value (P-value) less than or equal to 0.05 was considered significant.

## RESULTS

### Isolation and Identification of Strains

Data obtained microscopically, macroscopically, and via biochemical tests [using the Vitek2 system] indicated differences in the bacterial gut composition in normal individuals, obese individuals, and those that have undergone bariatric surgery. All bacteria isolated and identified in this study were found to belong to the Enterobacteriacea family (Table-1). The number of bacteria in the normal-weight group was greater relative to that identified in the obese and post-bariatric group (Fig.1), whereby abundance of *E. coli* was observed in patients that had undergone bariatric surgery (Fig. 2).

**Table-1:** Bacterial Species Isolated from Human Feces.

| Post-Bariatric Surgery | Obese | Normal Weight |
|---|---|---|
| Escherichia *coli* | Citrobacter *freundii* | Enterobacter *cloacae* |
| Pseudomonas *aeruginosa* | Serratia *marcescens* | Klebsiella *pneumonia* |
| Klebsiella *pneumonia* | Proteus *mirabilis* | Escherichia *coli* |
| Enterococcus *gallinarum* | Enterobacter *aerogenes* | |
| Serratia *odorifera* | Escherichia *coli* | |
| Gemella *morbillorum* | | |

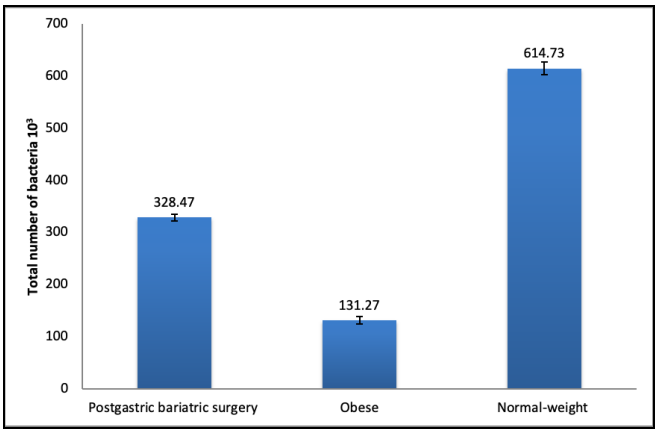


**Fig.1:** Total number of bacterial species isolated from human feces in the three study groups.

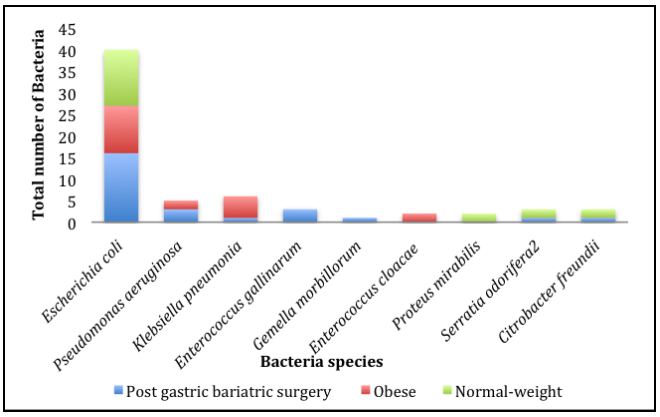


**Fig.2:** The number of bacterial species isolated from human feces in the three study groups.

Table-2 displays the means and SDs of different groups and their statistical significance. LDL was significantly higher in the obese and normal-weight groups ($p$ = 0.03 and $p$ = 0.05, respectively).

**Table-2:** Comparison between the Three Study Groups.

| | Groups | Mean | Standard Deviation | P-value |
|---|---|---|---|---|
| **Cholesterol** | 1 | 176.67 | 33.98 | 0.36 |
| | 2 | 165.33 | 26.11 | 0.42 |
| | 3 | 171.67 | 29.54 | 0.44 |
| **Triglyceride** | 1 | 95 | 43.37 | 0.23 |
| | 2 | 69.33 | 13.22 | 0.49 |
| | 3 | 69 | 14.9 | 0.23 |
| **HDL** | 1 | 62 | 11.43 | 0.32 |
| | 2 | 68.33 | 13.02 | 0.21 |
| | 3 | 57 | 12.0897 | 0.35 |
| **LDL** | 1 | 108 | 36.1201698 | 0.16 |
| | 2 | 137 | 5.89 | 0.002 |
| | 3 | 104.33 | 4.92 | 0.45 |

1 – Post-gastric bypass group, 2 – obese group, 3 – normal weight group, HDL – high density lipoprotein, LDL – low density lipoprotein, **P* is significant < 0.05.

## PCR Detection

E*. coli* strains identified using the Vitek2 system indicated a 220/258bp PCR product size observed on 1.5% agarose gel viewed with UV light (Fig.3).

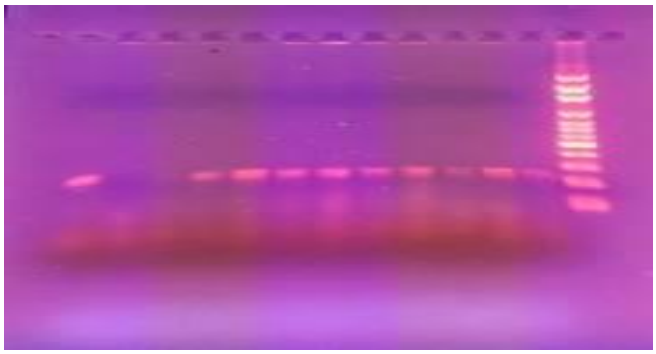

**Fig.3:** PCR product of isolates identified as E*.coli* viewed with UV light on 1.5% agarose gel. Lane 1 pertains to the positive control (E*. coli* (ATCC 25996), whereas Lane 2 – 13 relate to bacterial samples, andLane 14 denotes the DNA Ladder (sollis). Lane 2 and 3 failed to reveal the 220/258 bp bands.

## Antimicrobial Sensitivity Profile

The antimicrobial resistant strains of the E*. coli* isolated from Obese, normal-weight and post-gastric bariatric surgerygroup is shown in Table-3. Nine isolates obtained from subjects that have undergone bariatric surgery were sensitive to the tested antibiotics, 15 (93.8%) were resistant to amoxicillin; 10 (62.5%) were resistant to ampicillin andcefotetan; 7 (43.8%) were resistant to ofloxacin; 4 (25%) were resistant to cefotaxime, cefoxitin, and ciprofloxacin; and 3 (18.8%) were resistant to nitrofurantoin and trimethoprim/ sulfamethoxazole. Among the 11 isolates obtained from obese subjects, 3 (27.2%) were resistant to amoxicillin, 5 (45.5%) were resistant to ampicillin and cefotaxime; 2 (18.1%) were resistant to cefoxitin, cefixime, norfloxacin, andcefotetan; and 1 [9%] was resistant to ofloxacin (Table-3). Finally, among the 13 isolates obtained from the normal-weight group, 7 (53.8%) were resistant to amoxicillin and ampicillin; 5 (38.5%) were resistant to norfloxacin; 4 (30.8%) were resistant to cefotetan; 3 (23.1%) were resistant to cefotaxime; and 2 (15.4%) were resistant to cefoxitin, cefixime, gentamicin, and trimethoprim/ sulfamethoxazole (Table-3). None of the isolates was positive for ESBL (as determined by Vitek 2 system (Biome´rieux).

**Table-3:** Spread of Antimicrobial Resistance between the E. *coli* Strains Isolated from the three study groups.

| Antimicrobial | Resistance % | | | |
|---|---|---|---|---|
| | Post-Bariatric Surgery (*N*=16) | Obese (*N*=11) | Normal-Weight (*N*=13) | Total (*N*=40) |
| Amoxicillin | 93.75 | 27.28 | 53.85 | 62.5 |
| Ampicillin | 62.5 | 45.45 | 53.85 | 55 |
| Amoxicillin/ clavulanic acid | 0 | 0 | 7.69 | 2.5 |
| Ampicillin/sulbactam | 0 | 0 | 7.69 | 2.5 |
| cefotetan | 62.5 | 45.45 | 30.77 | 47.5 |
| Cefotaxime | 25 | 27.27 | 23.08 | 25 |
| Cefoxitin | 25 | 18.18 | 15.38 | 20 |
| Cefixime | 0 | 18.18 | 15.38 | 10 |
| Gentamicin | 0 | 0 | 15.38 | 5 |
| Ciprofloxacin | 25 | 0 | 0 | 10 |
| Norfloxacin | 0 | 18.18 | 38.46 | 17.5 |
| Ofloxacin | 43.75 | 9.09 | 0 | 17.5 |
| Nitrofurantoin | 18.75 | 0 | 0 | 7.5 |
| Trimethoprim/sulfamethoxazole | 18.75 | 0 | 15.39 | 12.5 |

## DISCUSSUION

Variation among the intestinal microbial enterotypes cannot be correlated to individual factors, like age or degree of obesity, geographical site, or short-term dietary amendments.[21] According to empirical evidence, long-term alimentation habits and certain clinical features seem to be stronger influencers on these gut microbial compositional differences.[22] Different authors have reported differences in gut microbiota

composition between obese and non-obese individuals.[7,23-26] Specifically, Guoand colleagues[9] observed a lower Bacteroidetes to Firmicutes ratio in obese subjects compared to normal-weight individuals. However, in their study Al-Assal and colleagues, andGuoand colleagues[7,9] found that Firmicutes were more prevalent than Bacteroidetes in the gut microbiota of obese participants. These incongruent findings suggest that new energy harvesting systems related to the metabolic capacity of Firmicutes could be present in the host human gut. However, as these results were not substantiated through subsequent investigations, further research on this subject is needed.In the studies conducted by Cotillard et al.[27] and Le Chatelier et al.[23], higher microbial richness compared to obese individuals was noted among normal-weight subjects [individuals with BMI in the normal range], which was attributed to their normal metabolic profile. Interestingly, findings yielded by both studies suggested that high levels of LDL [low-density lipoprotein] cholesterol increases the risk of heart disease and stroke tin both normal weight and obese subjects. Other authors have correlated the role of gut microbiota with LDL[28] suggesting that gut microbiota modification could help in the modulation of cardiovascular disease risk factors. Moreover, in the study conducted by Dao and colleagues,[29] individuals who had undergone bariatric surgery in order to lose excess weight showed variations in gut microbial composition depending on the type of gastric bypass surgery. In an earlier study, Furet et al.[24] demonstrated through 16Sq PCR measurements that, following the bypass surgery, F. *prausnitzii* increased in number regardless of diet. Later, Kong et al.[30] demonstrated via 16S pyrosequencing that microbial richness was greatly improved in patients that underwent RYGB, positing that half of this compositional difference was related to dietary intake. Other authors reported an increase in Proteobacteria after gastric bypass surgery.[31-33] Consistent with the findings yielded by previous studies,[11,30] the results obtained in the present work indicate the dominance of Proteobacteria, mainly E.*coli*, in the gut post-bariatric surgery, independent of the dietary intake, compared to normal-weight and obese participants.

The isolated E.*coli* were identified based on morphological characteristics, microscopic appearances, and a set of biochemical analysesthat were performed using Vitek2, and were further detected using a molecular technique, namely PCR amplification reaction, with *E.coli* (ATCC 25966) as the positive control (Fig.3). The results of this investigation indicated that stool samples obtained from normal-weight individuals had a higher bacterial diversity and quantity compared to the ob and gb groups (Fig. 1). In this study results are in agreement with those reported by other authors[11,33] suggesting that effective surgical bypass for morbid obesity has the capacity to significantly alter the stool microbial composition. In particular, the Gammaproteobacteria tends to increase, specifically members of the family [Enterobacteriaceae and E.*coli* in particular], which could be suggested as a marker of obesity variation post-surgery, independent of energy intake.[24] *E.coli* was also abundant in all three groups tested in the present study (Fig.1−3). Although the reasons behind this change are presently unclear, it could be attributed to the physiological and anatomical alterations in the gastrointestinal tract induced by the surgical bypass.[11,33] For instance, shortening the length of the small intestine and altering the oxygen content could favor the presence of facultative anaerobes of the Enterobacteriaceae family.[11,33] Therefore, these study highlight the impact on the gut microbiota in normal and obese subjects, as well as those that have undergone gastric bypass surgery. In order to minimize the antibiotic treatment effect on the bacterial intestinal composition. It was ensured that none of the participants had taken antibiotics for at least 12 months prior to the study. This criterion was imposed, given that antibiotics can drastically alter the types of bacteria present in the gut.[33]

It is also noteworthy thatthe present study was performed in Riyadh, Saudi Arabia, where 32.5% of the E*. coli* isolates were shown to be antimicrobial resistant. The most frequently observed antibiotic resistance pertained to amoxicillin and ampicillin, which was noted in three subjects under study. These results are consistent with those reported by Pires et al.[34] who found that ampicillin resistance was the most prevalent. Furthermore, Jakobsen et al.[35] found that human isolates had a higher resistance to ampicillin than to other drugs. In the present study, a high percentage of multidrug

resistance was also noted, particularly in isolates obtained from normal-weight subjects and those that have undergone bariatric surgery. One of the study objectives was to establish the resistance to E.*coli* in Saudi Arabia. We recommend the importance of a governmental program to monitor resistance development that could be associated with the increase in antibiotic consumption.

## CONCLUSION

In the extant studies, differences in the gut microbiota composition among obese patients, normal-weight individuals, and those that have undergone bariatric surgery are typically reported. The results presented in this workindicate that weight loss may induce partial recovery in bacterial components similar to that found in patients of normal weight.

## LIMITATIONS OF STUDY

One of the limitations of this study is its small sample size. A series of future studies are recommended with a larger sample size to establish significant correlations among various groups. Yet diet and other environmental factors were not carried out as a part of this study, therefore future research on this subject, it would be beneficial in this regard.

## ACKNOWLEDGMENT

This research project was funded by theDeanship of Scientific Research at Princess Nourah bint Abdulrahman Universitythrough the Fast-track Research Funding Program**.**

## CONFLICT OF INTEREST

None to declare.

## FINANCIAL DISCLOSURE

This research project was funded by theDeanship of Scientific Research at Princess Nourah bint Abdulrahman Universitythrough the Fast-track Research Funding Program**.**

## REFERENCES

1. Conterno L, Fava F, Viola R, Tuohy K. Obesity and the gut microbiota: does 91up-regulating colonic fermentation protect against obesity and metabolic disease? Genes Nutr 2011; 4 (6): 241-60.
2. OECD. Obesity and the economics of prevention: fit not fat. OECD Publishing, Paris. 2010: p. 23-44.
3. Romieu I, Dossus L, Barquera S, Blottière H, Franks P, Gunter M, et al. Energy balance and obesity: what are the main drivers? CCC. 2017; 28 (3): 247–58.
4. Cercato C. and Fonseca F. A. cardiovascular risk and obesity. Diabetol Metab Syndr. 2019; 11 (74): 1-15.
5. Blüher M. Obesity: global epidemiology and pathogenesis. Nat Rev. 2019; 15 (5): 288–98.
6. Jouret B, Ahluwalia N, Cristini C, Dupuy M, Negre-Pages L, Grandjean H, et al. Factors associated with overweight in preschool-age children in South Western France. Am J Clin Nutr. 2007; 85 (6): 1643-9.
7. Al-Assal K, Cristina A, Raquel M, Torrinhas S, Cardinelli C, Waitzberg D. Gut microbiota and obesity. Clin Nut Exp. 2018, 20 (11): 60-4.
8. Ridaura VK, Faith JJ, Rey FE, Cheng J, Duncan AE, Kau AL, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science. 2013; 341 (6150): 401-9.
9. Guo Y, Huang ZP, Liu CQ, Qi L, Sheng Y, Zou DJ. Modulation of the gut microbiome: a systematic review of the effect of bariatric surgery. Eur J Endocrin. 2018; 178 (1): 43–56.
10. Medina DA, Li T, Thomson P, Artacho A, Brocal V P, Moya A. Cross-regional view of functional and taxonomic microbiota composition in obesity and post-obesity treatment shows country specific microbial contribution. Front Microbiol. 2019; 10 (3): 234-9.
11. Peat C, Kleiman S, Bulik C, Carroll I. The intestinal microbiome in bariatric surgery patients. Eur Eat Disord Rev. 2015; 23 (6): 496-03.
12. Von Baum H, Marre R. Antimicrobial resistance of Escherichia *coli* and therapeutic implications. Int J Med Microbiol. 2005; 29 (5): 503-11.
13. Baumgartner M, Bayer F, Pfrunder-Cardozo KR, Buckling A, Hall AR. Resident microbial communities inhibit growth and antibiotic-resistance evolution of Escherichia coli in human gut microbiome samples. PLoS Biol. 2020; 18 (4): e3000465.
14. Smith D. Transfer of antibiotic resistance from animal and human strains of Escherichia coli to resident E.*coli* in the alimentary tract of man. Vet Rec. 1969; 141 (7607): 1174-6.
15. Seth EC, Taga ME. Nutrient cross-feeding in the microbial world. Front Microbiol. 2014; 5 (9): 1–6.

16. Klümper U, Recker M, Zhang L, Yin X, Zhang T, Buckling A, et al. Selection for antimicrobial resistance is reduced when embedded in a natural microbial community. ISME J. 2019; 13 (12): 2927-37.
17. Murray AK, Zhang L, Yin X, Zhang T, Buckling A, Snape J, et al. Novel insights into selection for antibiotic resistance in complex microbial communities. M Bio. 2018; 9 (4): 1–12.
18. Bengtsson-Palme J, Angelin M, Huss M, Kjellqvist S, Kristiansson E, Palmgren H, et al. The human gut microbiome as a transporter of antibiotic resistance genes between continents. Antimicrob Agents Chemother. 2015; 59 (10): 6551–60.
19. Meyer E, Lunke C, Kist M, Schwab F, Frank U. Antimicrobial resistance in Escherichia *coli* strains isolated from food, animals and humans in Germany. Infection. 2008; 36 (1): 59-61.
20. Gibson L, Khoury J. Storage and survival of bacteria by ultra-freeze. Lett Appl Microbiol. 1986; 3 (6): 127–9.
21. Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende D, et al. Enterotypes of the human gut microbiome. Nature. 2011; 473 (7346): 174-80.
22. Wu G, Chen J, Hoffmann C, Bittinger K, Chen Y, Keilbaugh S, et al. Linking long-term dietary patterns with gut microbial enterotypes. Science. 2011; 334 (6052): 105-08.
23. Le Chatelier E, Nielsen T, Qin J, Prifti E, Hildebrand F, Falony G, et al. Richness of human gut microbiome correlates with metabolic markers. Nature. 2013; 500 (7464): 541-6.
24. Furet J, Kong L, Tap J, Poitou C, Basdevant A, Bouillot J, et al. Differential adaptation of human gut microbiota to bariatric surgery-induced weight loss: links with metabolic and low-grade inflammation markers. Diabetes. 2010; 59 (12): 3049-57.
25. Munukka E, Wiklund P, Pekkala S, Völgyi E, Xu L, Cheng S, et al. Women with and without metabolic disorder differ in their gut microbiota composition. Obes. [Silver Spring].2012; 20 (5): 1082-7.
26. Turnbaugh P, Hamady M, Yatsunenko T, Cantarel B, Duncan A, Ley R, et al. A core gut microbiome in obese and lean twins. Nature. 2009; 457 (7228): 480-4.
27. Cotillard A, Kennedy S, Kong L, Prifti E, Pons N, Le Chatelier E, et al. Dietary intervention impact on gut microbial gene richness. Nature. 2013; 500 (7464): 585-8.
28. Dubenetzky M, Rasmussen H, Carr T, Walter J. The role of gut microbiota in the low-density lipoprotein [LDL] cholesterol-lowering effects of plant sterol esters. FASEB J. 2011; 25 (6):1-12.
29. Dao M, Everard A, Clement K, Cani P. Losing weight for a better health: Role for the gut microbiota. Clin Nut Exp. 2016; 6 (12): 39-58.
30. Kong L, Tap J, Aron-Wisnewsky J, Pelloux V, Basdevant A, Bouillot J, et al. Gut microbiota after gastric bypass in human obesity: increased richness and associations of bacterial genera with adipose tissue genes. Am J Clin Nutr. 2013; 98 (1): 16-24.
31. Graessler J, Qin Y, Zhong H, Zhang J, Licinio J, Wong M, Xu A, et al. Metagenomic sequencing of the human gut microbiome before and after bariatric surgery in obese patients with type 2 diabetes: correlation with inflammatory and metabolic parameters. Pharmacogenomics J. 2013; 13 (6): 514-22.
32. Ward E, Schuster D, Stowers K, Royse A, Ir D, Robertson C, et al. The effect of PPI use on human gut microbiota and weight loss in patients undergoing laparoscopic Roux-en-Y gastric bypass. Obes Surg. 2014; 24 (9): 1567-71.
33. Zhang H, DiBaise J, Zuccolo A, Kudrna D, Braidotti M, Yu Y, et al. Human gut microbiota in obesity and after gastric bypass. Proc Natl Acad Sci. 2009; 106 (7): 2365-70.
34. Pires M, Frota K, Martins J, Correia A, Cortez-Escalante J, et al. Prevalence and bacterial susceptibility of community acquired urinary tract infection in University Hospital of Brasília, 2001 to 2005. Rev Soc Bras Med Trop. 2007; 40 (7): 643-7.
35. Jakobsen L, Kurbasic A, Skjøt-Rasmussen L, Ejrnaes K, Porsbo L, Pedersen K, et al. Escherichia *coli* isolates from broiler chicken meat, broiler chickens, pork, and pigs share phylogroups and antimicrobial resistance with community-dwelling humans and patients with urinary tract infection. Foodborne Pathog Dis. 2010; 7 (5): 537-47.

### *Author's Contribution*

**KA:** Conception and design, drafting and critical revision for important intellectual content.

**NM:** Analysis of data, drafting of manuscript, and intellectual input.

**AM, AR, AO, AA, HA:** Analysis and interpretation of data.

**All Authors:** Approved the final version to be published.