

Prevalence and Antibiotic Susceptibility of Gram-Negative Bacteria Isolated from Different Meat Samples in Baghdad City

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ABSTRACT

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Background: The occurrence of Gram-negative (G -ve) bacteria in meat samples raises a major concern due to the possibility of drug resistance incidence since G -ve bacteria have built-in resistance mechanisms and can pass on genetic elements that enable other bacterial species to develop into drug-resistant as well. This drug resistance could be transferred to consumers through a food-borne route. This study aimed to evaluate the prevalence of Gram-negative bacteria in meat samples as well as to detect their antibiotic susceptibility patterns. **Materials and Methods:** For this purpose, 100 meat samples (ground meat, raw burgers, frozen chicken, and chicken carcasses) were collected, and obtained isolates were identified using conventional microbiological techniques including cultural and microscopic identification. After that antibiotic susceptibility patterns were detected using Kirby Bauer's disc diffusion method. **Results:** Results showed that 91 of the samples were harboring Gram-negative bacteria and *E.coli* was the most common isolate (51.64%) followed by *Klebsiella pneumoniae* (18.68%) while the least common isolate was each of *E. coli* O157:H7, *Aeromonas hydrophila*, *Kluyvera* spp., *Raoultella terrigena*, *Hafnia alvei*, and *Serratia marcescens* (1.10%). Susceptibility test showed that all isolates were susceptible to Meropenem and Imipenem while Ampicillin was the most resisted antibiotic. **Conclusions:** This study showed that meat samples harbor numerous pathogenic Gram-negative bacteria which showed antibiotic-resistant ability toward most tested drugs. However, Meropenem and Imipenem were the least resisted drugs, making them an appropriate choice for treating foodborne infections.

Keywords: Antibiotic susceptibility, Gram-negative bacteria, Imipenem, Meropenem

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1-INTRODUCTION

Food-borne infections have long been the leading cause of sickness and mortalities worldwide. As they impact both wellness and the economy, people are becoming more conscious of foodborne infections (1). Meat from healthy animals typically contains extremely few or no microbes, but contamination can occur during the killing, transporting, and processing processes (2). Meat is the flesh of animals produced by a variety of mammals that are utilized in the human diet. Skeletal muscles and their associated fat are referred to as meat, but it can also refer to other edible parts including body parts like skin, bone marrow, livers, brains, kidneys, or lungs (3). Meat is considered an ideal culture medium for the majority of microbes because of its high proportions of nitrogen-containing substances of various levels of complexity, high moisture, a plentiful supply of minerals, accessory growth factors, and some fermentable carbohydrates (glycogen) of a suitable pH (4). Like any food, meat can spread some infections, but this risk is diminished by thorough cooking and preventing cross-contamination (3). *Salmonella enterica*, a pathogen that causes illness, is frequently present in chickens. If safeguards are not implemented, disease-causing *Escherichia coli* O157:H7 that originates from the digestive system could infect minced beef during slaughter (5). It has been noted that while the animal's exterior and digestive system are the main sources of bacteria during slaughtering and butchering, other sources include blades, clothes, the environment, laborers, trucks, containers, and tools in general. Due to the large range of

organisms included, it may be expected that under normal circumstances, the majority of potential spoiling organisms are present and will be able to develop if favorable conditions arise (6). Antibiotics are frequently employed in the treatment of diseased humans and animals, as well as in the prevention and stimulation of growth in food-producing animals (7). Many studies have found that poor antibiotic selection and overuse can lead to resistance in diverse bacteria, making treatment of bacterial infections more challenging (8). The transmission of resistant bacteria to humans occurs through direct contact with animals, exposure to animal feces, ingestion of raw meat, and contact with meat surfaces (9). Due to the decreased effectiveness in treating infectious diseases, the growth of antibiotic resistance in bacteria is also becoming a public health threat (10). So this study aims to evaluate the prevalence of Gram-negative bacteria in meat samples and assess their susceptibility patterns toward frequently used antibiotics.

2- MATERIAL AND METHODS

SAMPLES COLLECTION AND CULTURING

From March to August 2021, a total of 100 meat samples, including 25 samples of each local and imported raw ground meat, raw burgers, parts of frozen chicken, and swabs from external surfaces of chicken carcasses, were collected from various local retail shops in Baghdad city, Iraq. Each sample was put in a sterilized bag, marked, placed in an icebox, and then transported to the laboratory for analysis. Samples processing was according to (11). Each sample was cultured on MacConkey agar (Oxoid, UK) before incubation for 24 h at 37 °C.

MICROSCOPICAL AND CULTURAL EXAMINATIONS

Grown colonies were described for their appearance, size, color, and texture after incubation. A smear was taken from a colony and placed on a glass slide for Gram staining before cells were examined under the compound microscope. Results were described according to (12).

Identification of isolates

Biochemical analysis was used to identify *Enterobacteriaceae* and other Gram-negative bacteria by using the analytical profile index (API) strips (BioMérieux, France) then further identification was carried out using Vitek 2 system (BioMérieux, France) results were read according to (13,14).

Antibiotics susceptibility test

According to the Manual on Antimicrobial Susceptibility Testing, Kirby Bauer's disc diffusion technique was used to assess antibiotic susceptibility in Gram-negative bacteria (15). Bacterial colonies were grown in Muller Hinton broth at 37 °C for 24 hours. Then, they were diluted to 0.5 McFarland standards. The obtained results were compared to those of the "Clinical Laboratory Standards Institute" (CLSI) (2020). The used antibiotics were Meropenem (Mem), Ampicillin (Am), Clarithromycin (CLR), Imipenem (IMP), Amoxicillin/clavulanic acid (AMC), Ciprofloxacin (CIP), Streptomycin (S), and Gentamicin (GM).

3-RESULTS

From a total of 100 meat samples, 91 (91%) Gram-negative bacteria were isolated. Their microscopic and cultural morphology is described in Table (1).

Table (1): Microscopic and cultural features of Gram-negative bacteria isolated from different meat samples.

Suspected isolate	Number	Microscopic examination	Cultural examination
<i>Escherichia</i> spp	47	Rod-shaped cells with rounded ends	Non-mucoid, lactose fermenters on MacConkey agar
<i>E. coli</i> O157:H7	1	Rod-shaped cells with rounded ends	Slightly translucent, almost colorless, with a diameter of 1 mm, and has a faint pale brownish look on sorbitol MacConkey agar which indicates its inability to ferment sorbitol
<i>Klebsiella</i> spp	17	Rod-shaped	Large dome-shaped highly viscous, lactose fermenters on MacConkey agar
<i>Salmonella</i> spp	7	Rod-shaped	Non-lactose fermenters colorless on MacConkey agar
<i>Enterobacter</i> spp	5	Rod-shaped with rounded ends	Large, sticky, pale in color on MacConkey agar
<i>Serratia</i> spp	4	Rod-shaped	2-3 mm in diameter, convex, non-lactose fermenters, mucoid, and non-pigmented on MacConkey agar
<i>Pseudomonas</i> spp	3	Rod-shaped	Smooth, translucent, large, low convex, 2-4 mm in diameter with an irregular spreading edge on MacConkey agar
<i>Citrobacter</i> spp	3	Rod-shaped	Dark pink and smooth colonies on MacConkey agar
<i>Aeromonas</i> spp	1	Straight bacilli, singles, pairs, or rarely short chains, and non-spore-forming	2-3 mm diameter with a pale shape on MacConkey agar
<i>Kluyvera</i> spp	1	Rod-shaped	Pink round-shaped colonies on MacConkey agar
<i>Raoultella</i> spp	1	Rod-shaped	Lactose fermenter-producing mucoid colonies on MacConkey agar
<i>Hafnia</i> spp	1	Rod-shaped	Large, smooth, convex, and pink or translucent colonies of 2-3 mm in diameter with entire edges on MacConkey agar

After cultural and microscopic identification, the results were confirmed by the API system and Vitek 2 system as shown in Table (2).

Table (2): Number and percentage of bacterial isolates

Isolate	No. of isolate	Percentage %
<i>E. coli</i>	47	51.64
<i>Klebsiella pneumoniae</i>	17	18.68
<i>Salmonella spp</i>	7	7.69
<i>Enterobacter coloaecae</i>	5	5.49
<i>Citrobacter freundii</i>	3	3.30
<i>Serratia liquefaciens</i>	3	3.30
<i>Pseudomonas aeruginosa</i>	3	3.30
<i>E. coli</i> O157:H7	1	1.10
<i>Aeromonas hydrophila</i>	1	1.10
<i>Kluyvera spp.</i>	1	1.10
<i>Raoultella terrigena</i>	1	1.10
<i>Hafnia alvei</i>	1	1.10
<i>Serratia marcescens</i>	1	1.10
total	91	100.0

After isolate identification, they were subjected to antibiotics susceptibility testing using the disc diffusion technique as described above. The results indicated that Meropenem and Imipenem were the most effective antibiotics against isolates since all isolates were susceptible to these two antibiotics while 3 species were intermediate resistant to ciprofloxacin when compared to CLSI as illustrated by (Figure 1). On the other hand, the most resisted antibiotic by isolates was ampicillin with 100% resistance. *Serratia liquefaciens* and *Citrobacter freundii* were the most resistant isolates since they showed resistant to four different antibiotics (Am, AMC, S, and GM) intermediate to CLR and CIP and susceptible to only two antibiotics (MEM and IPM) (Figures 2 & 4). On the contrary, *Salmonella choleraesuis arizonae* was the least resistant bacteria when it was sensitive to four antibiotics and intermediate to two while resistant to only two antibiotics (Figure 2). The results also showed (Figure 3) that *Raoultella terrigena* showed the most intermediate antibiotic profile when four antibiotics (CLR, AMC, CIP, and S) showed an intermediate effect when compared to CLSI guidelines.

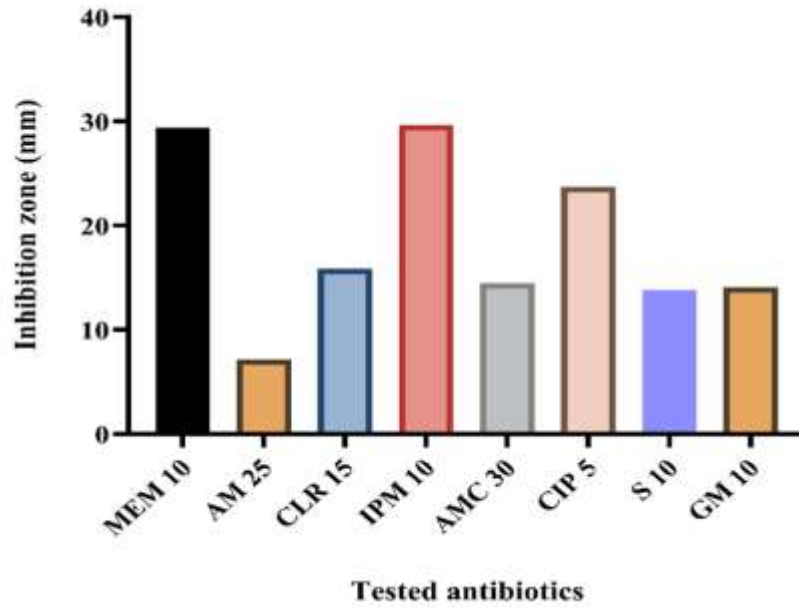


Figure (1): Most effective antibiotics against Gram-negative bacteria isolated from meat samples

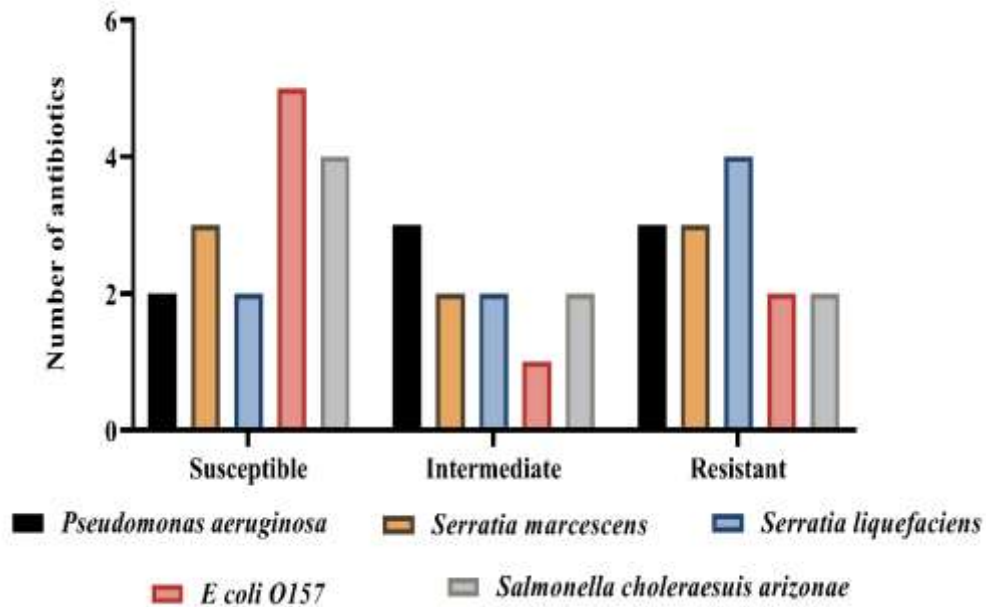


Figure (2): Antibiotics profile of *Pseudomonas aeruginosa*, *Serratia marcescens*, *Serratia liquefaciens*, *E. coli O157*, and *Salmonella choleraesuis arizonae* isolated from different meat samples

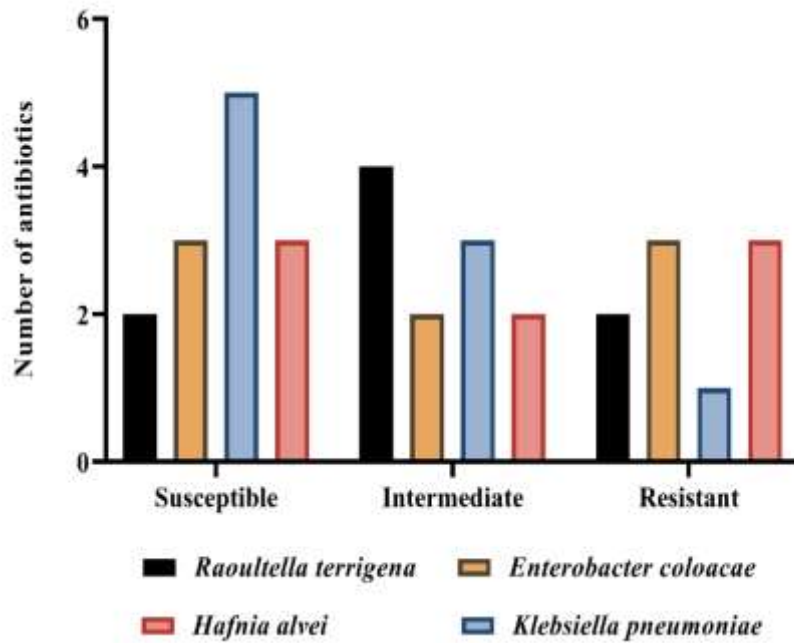


Figure (3): Antibiotics profile of *Raoultella terrigena*, *Enterobacter coloaecae*, *Hafnia alvei*, and *Klebsiella pneumoniae* isolated from different meat samples

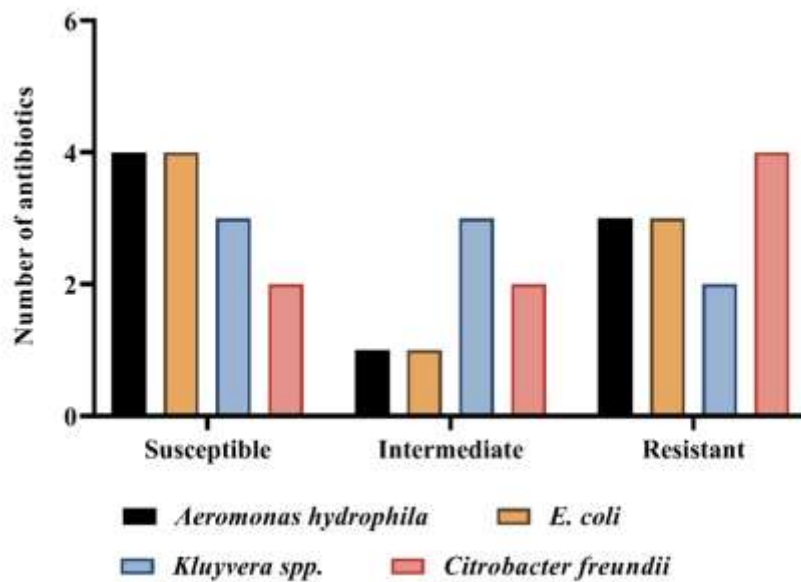


Figure (4): Antibiotics profile of *Aeromonas hydrophila*, *E. coli*, *Kluyvera spp.*, and *Citrobacter freundii* isolated from meat samples

4-DISCUSSION

Out of 100 meat samples 91 Gram-negative bacteria were detected which agrees with several previous studies (16, 17). Increased prevalence rates could be brought about by incorrect handling, poor cleaning, unsatisfactory processing, and post-processing contaminants from the polluted environment (1). The most common species was *E. coli* 48 (52%) followed by *K.pneumoniae* 17 (18%) and then by *S. choleraesuis arizonae* 7 (7%). This could be owing to the use of contaminated water during slaughtering, washing, and other processing operations. Also, blood is considered a very rich medium for the growth of various types of bacteria, in addition to incorrect storage processes as well as *E. coli* being a common resident of animal and human digestive systems (17). The ability of bacteria to escape being destroyed is attributed to their antibiotic resistance comes from antibiotic-resistance genes that are already present in the microbe's genetic makeup or can be acquired by plasmids from other bacteria. This resistance increases the efforts to treat infections. Thus, antibiotic resistance is one of the greatest challenges in modern medicine. In terms of antibiotic residues, meat also contributes significantly to the spread of antibiotic-resistant genes (1). Results of this study displayed that the majority of isolates were entirely sensitive to meropenem and imipenem, and to a lesser level susceptible to ciprofloxacin, as shown in Figure 1. Similarly, in a study conducted by (18), all Gram-negative bacteria identified showed sensitivity to meropenem and imipenem. Close susceptibility patterns were reported by (19, 20) when no resistance was detected to meropenem or imipenem by Gram-negative bacteria isolated from meat samples. The obtained results suggest that ciprofloxacin is still an appropriate drug to use against invasive infections caused by Gram-negative bacteria since none of the examined isolates developed resistance to it, despite a small number of isolates exhibiting intermediate susceptibility. Furthermore, since all isolates were susceptible to monobactams (meropenem and imipenem) they can be considered as alternative drugs for more resistant bacteria. This result may be attributed to the limited prescription of meropenem and imipenem. Yet, their poisonous nature and increased harm to microflora should not be disregarded, It was also found that ampicillin was the most resistant drug when all tested isolates showed a resistant pattern toward it. This result comes in agreement with the study conducted by (21) when they showed that *E. coli* and *Klebsiella* spp isolated from Ready-to-Eat Street Foods possessed high resistance to ampicillin. Also, another research (22) found that *E. coli* obtained from samples of chicken meat was highly resistant to ampicillin. This may be due to the ability of pathogenic isolates to harbor β -lactamase enzyme which can play a major role in their resistance. This study has certain limitations. First, it is explained by the comparatively small sample size and second, by the small regions chosen for sample collection, hence it's possible that the outcomes cannot be applied to other sites. Future research with a larger sample size from a wider regional selection will give a more complete picture of the Gram-negative bacteria contaminating meats.

5- CONCLUSIONS

The prevalence of pathogenic Gram-negative bacteria in meats is relatively diverse and high. These pathogens showed antibiotic resistance toward several drugs which present health risks to consumers. It is clear from this study that Meropenem and Imipenem are the most effective antibiotics against isolated pathogenic bacteria while Ampicillin is the most resisted drug. In addition, *E. coli* was the most prominent isolate while *A. hydrophila*, *Kluyvera* spp., *R. terrigena*, *H.alvei*, and *S.marcescens* were the least prevalent isolates.

Conflict of Interest

The authors have no financial conflicts of interest to declare.

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انتشار وجود البكتريا السالبة لصبغة غرام المعزولة من عينات لحوم مختلفة في مدينة بغداد وفحص حساسيتها للمضادات الحيوية

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الخلاصة

خلفية عن البحث: وجود البكتريا السالبة لصبغة غرام في عينات اللحوم يثير مصدر قلق كبير لاحتمالية وجود مقاومة للمضادات الحيوية بما ان البكتريا السالبة لصبغة غرام تمتلك الليات مقاومة مضادات حيوية ذاتية ممكن انتقالها جينيا لأنواع بكتيرية أخرى مما تمكنها من مقاومة المضادات هي الأخرى. مقاومة المضادات هذه ممكن انتقالها للمستهلك عن طريق الغذاء. **الهدف:** هدفت هذه الدراسة لتقييم انتشار البكتريا السالبة لصبغة غرام في عينات اللحوم بالإضافة الى فحص أنماط حساسيتها للمضادات الحيوية. **المواد وطرق العمل:** لهذا الغرض تم جمع 100 عينة لحوم (لحم مفروم و أقراص اللحم المفروم غير المطبوخ و دجاج مجمد و أجساد الدجاج) و تم تشخيص العزلات المتحصلة بتقنيات الاحياء المجهرية التقليدية المتضمنة التشخيص الزراعي و المجهري. بعد ذلك تم التحري عن أنماط حساسية المضادات الحيوية باستخدام طريقة انتشار القرص **Kirby Bauer**. **النتائج:** أظهرت النتائج ان 91 عينة كانت تحتوي على البكتريا السالبة لصبغة غرام و كانت *E. coli* الأكثر شيوعا (51.64%) ثم *Klebsiella pneumoniae* (18.68%) بينما الأقل شيوعا كانت كل من *Serratia marcescens* و *Aeromonas hydrophila* و *Kluyvera spp* و *Raoultella terrigena* و *Hafnia alvei* و *Serratia marcescens* (1.10%). فحص حساسية المضادات الحيوية اظهر ان جميع العزلات كانت حساسة للمضادين الحيويين **Meropenem** و **Imipenem** بينما كان **Ampicillin** الأكثر مقاومة. **الاستنتاج:** نستنتج من ذلك ان عينات اللحوم تحتوي على العديد من البكتريا السالبة لصبغة غرام المرضية والتي أظهرت قابلية لمقاومة المضادات الحيوية المستخدمة. على الرغم من ذلك فإن **Meropenem** و **Imipenem** كانا الأقل مقاومة مما يجعلهما الاختيار المناسب لمعالجة العدوى المنقولة بالغذاء.

الكلمات المفتاحية: حساسية المضادات، البكتريا السالبة لصبغة غرام، Imipenem، Meropenem.