Isolation, Identification and Sensitivity of Escherichia Coli from Fresh Vegetables in Zawia and Surman City, Libya

عزل وتشخيص وحساسية بكتيريا الاشريكية القولونية من الخضروات الطازجة في مدينتي الزاوية وصرمان الليبية

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الملخص:

تستخدم الخضروات الجاهزة للأكل في المطاعم وفي البيوت في ليبيا ومن هنا تأتي أهمية فحصها للتأكد من عدم تلوثها ببكتيريا الاشريكية القولونية.

تهدف هذه الدراسة لعزل بكتيريا الاشريكية القولونية من الخضروات الجاهزة للأكل كما تهدف الى معرفة حساسة البكتيريا لستة من المضادات الحيوية على هذه البكتيريا.

ثلاثة عينات تشمل (السلطة والجرجير والبقدونس) ثم تجميعها من مدينتي الزاوية وصرمان وتم استخدام وسطين غذائيين هما الماكونكي والنيترنت اجار وفحصت العينات لتحديد وجود بكتيريا الاشريكية القولونية ولتعريف هذه البكتيريا ومعرفة خواصها تم باستعمال اختبار بايوكيميائي (ايه بي أي 20 اي) كما ثم استخدام أقراص المضادات الحيوية حسب طريقة (كيربي و باور) لتحديد حساسية البكتيريا للمضادات الحيوية.

خمسة وثلاثون عزلة وهو ما يشكل خمسة وثمانون بالمائة كانت موجبة لوجود البكتيريا الاشريكية القولونية, واكبر نسبة كانت موجودة في الجرجير بنسبة اثنان واربعون فاصلة ست وثمانون بالمائة متبوعا بالخس والسلطة بنسبة ثمان وعشرون فاصلة سبعة وخمسون بالمائة, النسبة الأكبر من العزلات الملوثة بهذه البكتيريا كانت بمدينة الزاوية بنسبة واحد وسبعون فاصلة أربعة بالمائة في حين كانت بمدينة صرمان ثمان وعشرون فاصلة ستة بالمائة , كل العزلات كانت مقاومة للمضاد الحيوي الاوقمنتين في حين خمس عزلات فقط وتمثل نسبة اربعة عشر فاصلة تسع وعشرون بالمائة كانت مقاومة للمضاد الحيوي الاميكسين وهذا يمكن ان يشكل خطرا على الصحة العامة.

الكلمات المفتاحية: حساسية المضادات الحيوية، الاشريكية القولونية، الخضروات الجاهزة للأكل.

Abstract

In Libya, ready to eat (RTE) ***vegetables*** are most used at restaurants and home. Therefore, it is very important to examine possible ***Escherichia coli (E. coli)*** contamination in RTE vegetables. This study aimed to isolate E. coli from RTE Vegetables. The antimicrobial sensitivity of ***E. coli*** obtained from RTE vegetables was as well performed using 6 usually used antibiotics. Three samples (Lettuce, watercress, and parsley) were collected from Zawia and

Surman cities, using media of MacConkey agar and nutrient agar. These samples were analyzed to detect the presence of E. coli. Identification and characterization of E. coli isolates were performed using analytical profile index ABI 20 E. Using the Kirby-Bauer disc diffusion method, antimicrobial susceptibility was determined. Thirty-five (58.3%) of samples showed a positive result of the presence of E. coli, highest percentage (42.86%) was isolated from watercress followed by parsley and lettuce (28.57%) for each one. Highest percentage (71.4%) was isolated from Zawia City, while the lowest percentage (28.6%) was isolated from Surman City. The findings revealed that 35 isolates (100%) were resistant to Augmentin and five isolates (14.29%) were resistant to Amikacin as well.

This revealed that 58.3% of RTE vegetable samples were contaminated with E. coli and the isolates highly resistant to Augmentin (100%) could be hazardous for community health.
Keywords: Antimicrobial sensitivity, Escherichia coli, Ready to eat vegetable.

1. Introduction

Ready-to-eat (RTE) vegetables such as Lettuce, watercress and parsley are fresh products subjected to low processing to preserve their freshness. Their production technique is very simple and does not require severe treatments or the use of preservatives (Ragaert et al., 2007, p171).

The RTE vegetables are popular around the world as they are an essential source of vitamins, nutrients, and fiber for people. Consumption of fresh vegetables has been linked to an increase in outbreaks of foodborne illnesses (Callejon et al., 2015, p32).

The process of producing fresh produce is complex and includes numerous important steps where microbiological safety may be impaired. As a result, microbial contamination may occur at any step in the farm-to-consumer supply chain (production, harvest, fresh-cut processing, wholesale storage, transportation, or retailing and handling in the home), and it may occur from a wide range of sources, including the environment, animals, or people [FAO/WHO, 2010].

Escherichia coli (E. coli) is a gram negative bacteria that belong to the Enterobacteriaceae family (Gritli et al., 2015, p899) and has been isolated from vegetables, including Lettuce, watercress and parsley ***(***Abakpa et al., 2018***, p80***, Painter et al., 2013, p407***)***. E. coli contamination of fresh produce may occur from water and soil [Chekabab et al., 2013, p1, Solomon et al., 2002, p397]. In most developing countries, including Libya, there is usually inadequate oversight and examination of E. coli levels in food that sold in the market, particularly salad vegetables. This could be harmful, especially if the people are unaware of the situation. Several studies have demonstrated presence of pathogenic E. coli in fresh vegetables and fruits (Kim et al., 2014, p.367, Moses et al., 2016, p22).

Antibiotic resistance in pathogenic and commensal microorganisms has emerged as one of the most serious global health issues of the twenty-first century. Antibiotic resistance is common in bacterial isolates from developing nations as a result of antibiotic abuse and misuse (Bailey et al.,2010, p9, Chantziaras et al., 2014, p.225, Schroeder et al., 2017).

Commensal E. coli inhabits the intestines of many mammals, including humans, and serves as a possible reservoir for antimicrobial resistance genes, as well as playing an important role in the ecology of antimicrobial resistance in bacterial populations. The incidence of resistance in commensal E. coli is a potential indication of antibiotic resistance in community bacteria (Phongpaichit et al., 2008, p279, Al-Saedi et al., 2017, p1).

2. **Methodology**

The study was carried out from March to July of 2022 and the analysis of isolates took place at Surman Technology University Laboratory, Surman City, Libya.

2.1 Sample collection and microbiological analysis of vegetables.

Sixty samples of RTE vegetables including Lettuce, watercress, and parsley were aseptically collected from different places, traditional market in Zawia and Surman Cities, Libya. They were then aseptically chopped into smaller pieces using a sterile scalpel. Each 25 grams of the leafy vegetable samples was transferred aseptically to 225 ml of sterile buffered peptone water (BPW) 0.1% (w/v) using sterilized forceps and shacked for 3 min by a shaker (Shalini, 2010). Serial dilutions were prepared in normal saline (0.85% NaCl) from original homogenized, and then the appropriate dilutions (in duplicate) were placed on MacConkey agar to identify the lactose fermenting colonies. A portion of an isolated colony was streaked on nutrient agar and aerobically incubated at 37 °C for 24 hours to obtain pure cultures.

2.2 Identification of E. coli isolates using analytical profile index ABI 20 E.

API 20E was used as a standardized identification system for Enterobacteriaceae and other Gram-negative rods (Salah et al., 2019, p8, Wayne, 2005). To identify E. coli, pure E. coli isolates were sub-cultured on nutrient agar and incubated at 37°C for 18-24 hours. Isolates were identified using the numerical profile of API 20E that was observed.

2.3 Antibiotic Susceptibility test

All isolates were tested for antibiotic susceptibility using the Kirby-Bauer disc diffusion method. Ceftriaxone 30 g (CRO), Amikacin 30 g (AK), Augmentin (amoxicillin + clavulanic acid) 20/10 g (AMC), Levofloxacin 5 g (LEV), Ciprofloxacin 5 g (CIP), and Imipenem 10 g (IPM) are examples of regularly used antibiotics. The susceptibility test was performed using Mueller-Hinton agar and McFarland 0.5 from overnight cultures, followed by an incubation period of 24 hours at 37 °C. After incubation, inhibition zone diameters were determined with a millimetric ruler. CLSI guidelines (Soriano et al., 2006) were used to interpret the inhibition zone.

3. Results

3.1. Isolation and characterization

Lactose and non-lactose fermenting E. coli colonies were grown on MacConkey agar. Lactose-fermenting isolates formed pink to red colonies. Non-lactose fermentation colonies were colorless.

3.2. Results of using API 20 E in identification

Using the API 20 E method, 35 (58.3%) of the isolates were identified as E. coli (Table 1).

Table 1. Distribution of according to API 20 E.

Sample	No. of examined samples	No. of positive	% of positive
Lettuce	10	10	100
watercress	15	15	100
parsley	10	10	100

3.3. Prevalence of E. coli

A total of 35 (58.3%) of positive results for E. coli were isolated from 60 samples of RTE vegetables, highest percentage (42.86%) was isolated from watercress followed by parsley and lettuce (28.57%) for each one (Fig. 1). Highest percentage (71.4%) was isolated from Zawia City, while the lowest percentage (28.6%) was isolated from Surman City (Fig. 1).

Fig. 1: Number of E. coli isolated from different vegetables and places.

3.4. Antibiotic susceptibility test

Susceptibility tests of the 35 E. coli isolates revealed that all of them were Augmentin resistant (100%). Five isolates (14.29%) were also resistant to Amikacin. All E. coli isolates showed susceptible to Ceftriaxone, Levofloxacin, ***Ciprofloxacin*** and Imipenem (100%) (Fig. 2).

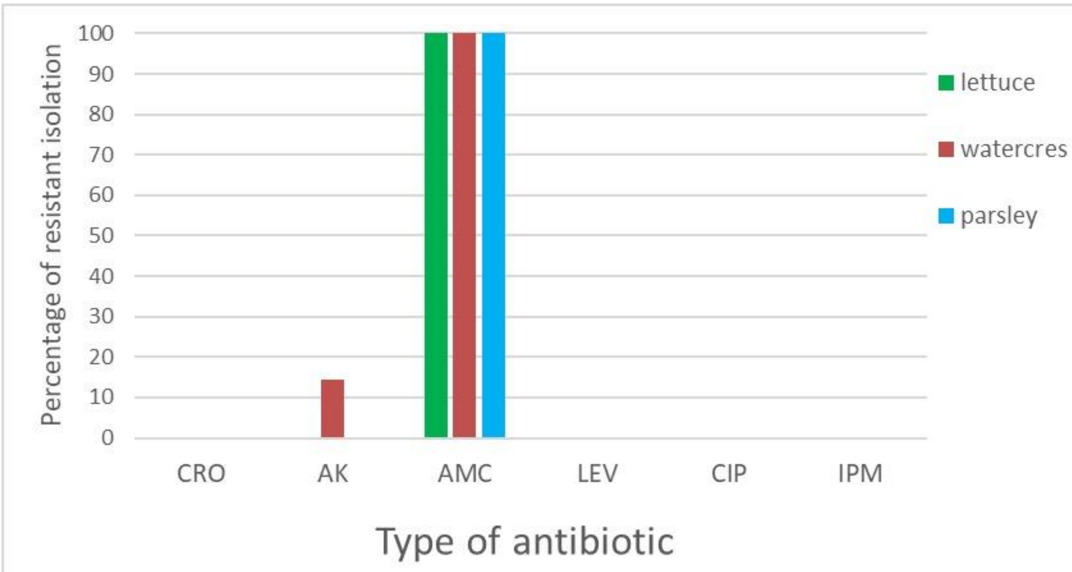

Fig. 2: A graph showing percentage resistance of ***E. coli*** isolates.

4. Discussion

Fresh vegetables can act as a major source and vehicle for the transmission of foodborne disease despite their nutritional and health benefits because they are typically exposed to microbial contamination throughout the supply chain, from the stage of production on the farm to the stage of selling in the market, even at the serving preparation stage by consumers before they consume it (Atwill et al., 2015, p240). The microbial contamination of fruits and vegetables can result from a variety of sources. Direct or indirect microbial contamination can happen during pre-harvest, harvesting, and post-harvest handling.

The present study revealed that 35 out of 60 RTE Vegetables samples were contaminated with E. coli (58.3%). Watercress showed the high contamination (42.86%), followed by parsley and lettuce (28.57%) (Fig. 1). Watercress is mostly consumed in raw forms in salad cuisines and therefore have higher risk of microbial transmission and health hazard to the community.

The fact that leafy vegetables are typically produced and harvested near to the soil may be the cause of this high bacterial level. As a result, the crops might be more readily exposed to contaminated soil, irrigation water, manure, and associated soil splash (Avcioglu et al., 2011, p189).

Contrary to those with smooth surfaces, leafy vegetables and/or vegetables with uneven surfaces make it easier for E. coli colonies to attach to the surface on the farm or when washed with contaminated water (Ayed et al., 2009, p393, Alemu et al., 2019, p410).

The rate of contamination by E. coli (58.3%) found in the present study was higher than those reported by (Sagoo et al., 2003, p403) for vegetable salads from United Kingdom (1.3%), by (Abadias et al., 2008, p121) for samples of fresh chopped vegetables in Spain (11.4%) and by (Prado et al., 2008, p221) in Brazilian samples of minimally processed vegetables. However, in contrast, (Srisamran et al., 2022, p13) found a similar prevalence of E. coli contamination in conventional and organic fruits and vegetables that were sold in retail marketplaces [56.1%].

The current analysis revealed that watercress (42.86%) showed a high contamination level and recorded greater rates, due to its irregular surfaces, which enable bacteria attach to it more readily.

Several Egyptian investigations obtained findings that different form the current study. Watercress had a 55.7% E. coli contamination rate, according to (Etewa et al., 2017, p192) in the Sharkia Governorate. The most contaminated vegetable, according to (Eraky et al., 2014, p7) in the Qualyobia Governorate, found that lettuce was the most contaminant vegetable (45.5%), followed by watercress (41.3%). On the other hand, (Hassan et al., 2012, p500) from a study in Alexandria found lower rates of the prevalence of E. coli (11.2%).

It is known that antibiotic-resistant bacteria or the resistance genes that control them may spread from animals to people through the food chain (Boehme et al., 2004, p522). Since epiphytic bacteria can develop antibiotic resistance as a result of the frequent use of antibiotics in agriculture, soil treated with organic fertilizers like sewage sludge and manure, and contaminated irrigation water, raw vegetables may play a role in this phenomenon. These elements could result in the contamination of plants with resistant bacteria from human or animal sources (De la Cruz & Davies, 2000, p128, Boehme et al., 2004, p522).

From antimicrobial susceptibility testing of 35 E. coli isolates, 35(100%) were resistant to Augmentin, 5 (14.29%) were resistant to Amikacin, 30 isolates (85.71%) were susceptible to Ceftriaxone, Levofloxacin, ***Ciprofloxacin and*** Imipenem (Fig. 2). These results were similar to that documented by (Poudel et al., 2020, p88) who reported that 94% of E. coli isolates from raw salad vegetables were resistant to amoxicillin + clavulanic acid. These results were similar to that documented by (Poudel et al., 2020, p88) who reported that 94% of E. coli isolates from raw salad vegetables were resistant to amoxicillin + clavulanic acid. On the contrary, according to (Marwa et al., 2012, p176) the majority of E. coli isolates from food were sensitive Augmentin.

Due to constant use of antibiotics by humans, there is an increasing resistance to them, veterinary and agriculture medicine which creates certain stresses that support the existence of antibiotic resistant bacteria (Heuer & Smalla, 2007, p3).

Conclusion

Out of the 60 RTE vegetable samples examined in this study, 58.3% of the vegetables were contaminated with E. coli and some isolates were found to be resistant to multi-drugs and presented high risk factors for public health. Contamination itself reflects poor hygiene quality that may be related to substandard agriculture, handling, packaging, and marketing practices. Better monitoring programs are necessary to prevent the potential epidemics as fresh perishables are now substantially linked to bacterial infection.

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