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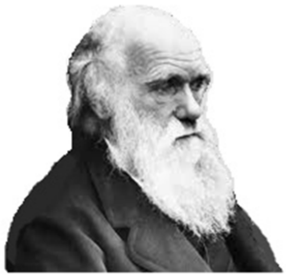

World News of Natural Sciences

An International Scientific Journal

WNOFNS 24 (2019) 365-371 EISSN 2543-5426

Antibiotic Susceptibility Test of Bacteria Isolated From Fruit Juices Sold in Cafes and Restaurants of Debre-Markos Town, North Western Ethiopia

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ABSTRACT

The prevalence of antimicrobial resistance, among food pathogens, has increased during recent decades. In this work, pathogenic bacteria, such as *Staphylococcus aureus*, *Salmonella* spp., *Shigella* spp., and *E. coli* were isolated following standard methods. The bacterial isolates were then tested for their sensitivity to common antibiotics using the disc diffusion method on Mueller-Hinton Agar. All of the pathogenic bacteria were found to be resistant to erythromycin and almost all were sensitive to penicillin.

***Keywords*:** Antibiotics, Antimicrobial resistance, Debre Markos, Fruit juices, Pathogens, *Salmonella, Shigella, E. coli, Staphylococcus aureus, B. cereus, Klebsiella, Enterobacter, Ps. aeruginosa*

1. INTRODUCTION

The antimicrobial resistance of bacteria isolated from food and other sources, against commonly used antibiotics has increased from time to time (Vicas and Singh, 2010). Not only their presence, but also their resistance to the commonly used antibiotics has become a concern for consumers.

Some reports have revealed that antibiotic resistance levels are becoming elevated among food-borne pathogens, such as *Salmonella* and *Shigella* (Mache, 2002). Although it is difficult

(Received 29 April 2019; Accepted 15 May; Date of Publication 16 May 2019)

to prove a direct role of drug resistance in bacteria contaminating food items with increased clinical cases of resistant infections, the presence of such bacteria in food items could play a role in the spread of antimicrobial resistance amongst food-borne pathogens (Farzana *et al.*, 2009). The incidence of resistant bacteria in foodstuff is a worldwide phenomenon. It is a major public health threat (Khan and Malik, 2001) as these organisms have been isolated from wide range of foodstuffs consumed by human.

The prevalence of antimicrobial resistance among food pathogens has increased during recent decades (Davis *et al.*, 1999; Garau *et al.*, 1999; Threfall *et al.*, 2000 and Chui *et al.*, 2002). Despite the extensive studies, the antibiotic sensitivity of theses bacteria has not been well studied, especially in a developing country. The aim of this research is therefore to test the antibiotic susceptibility of bacterial pathogens to some of the commonly prescribed antibiotics in the study area.

2. MATERIAL AND METHODS

Thirty-six samples of avocado and mango of locally prepared unpasteurized fruit juices were collected from six cafe or restaurant in Debre-Markos town from February 2014 to May 2014. 250 mL of juice samples were collected from cafes, in sterile beakers aseptically labeled, and immediately transported to Debre-Markos University Laboratory in an icebox where they were processed immediately.

Pathogenic bacteria such as *Salmonella*, *Shigella, E. coli, S. aureus, Klebsiella* spp and *B. cerues* were isolated according to the procedures outlined by Food and Drug Administration (FDA) (2001). For detecting, the presence of *Salmonella* and *Shigella,* 25 mL of juice sample was added into 225 mL of sterile peptone water and homogenized by shaking. The resulting dilution was then serially diluted up to 10^{-5} dilution. From the 10^{-1} and 10^{-2} dilutions, 1 mL was taken and inoculated into tubes of Lactose broth and incubated at 37 ºC for 48 hours. A loopful of sample from each culture was then transferred to Rappaport Vassiliadis (RV) broth and incubated at 42 ±0.2 °C for 24 hours in water bath. Positive samples were confirmed by streaking on Hektoen Enteric Agar and then biochemically tested for the presence of *Salmonella* and *Shigella.* In all cases, for confirmation of the pathogens, typical colonies were identified based on cultural, microscopic and biochemical characteristics (Buchanan and Gibbons NE, 1974).

All isolates of pathogenic bacteria were tested for their sensitivity to antibiotics by means of the disc diffusion method on Mueller-Hinton Agar (Difco, Detroit, MI) as described previously by Bauer *et al*. (1966)) using *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 as standard obtained from Amhara Regional State Centeral Laboratory. All disks used in the disk diffusion test were obtained from BECTON, USA, in the following concentrations: Ampicillin (Amp 10 μg), Amoxicillin (Amx 10 μg), Gentamycin (Gm 10 μg), Chloramphenicol (C 30 μg), Streptomycin (S 10 μg), Penicillin G (P 5 Units), Tetracycline (Te 30 μg) and Erythromycin (Er 2 μg). Briefly, five colonies of each isolate were introduced into 5 mL of Nutrient broth, incubated for 4 hours, and the culture turbidity was adjusted to a 0.5 McFarland standard. Sterile cotton swab was dipped into the suspension and spread evenly over the entire Mueller-Hinton Agar surface.

The antibiotics impregnated discs were then placed onto the surface of the inoculated plates and incubated at 37 ºC for 16-18 hours. After incubation, diameters of the zones of inhibition were measured in mm and interpreted as susceptible, intermediate, and resistant (CLSI, 2007).

3. RESULT AND DISCUSSION

According to current study, all the juice samples tested were devoided of *Salmonella* and *Shigella*. The prevalence of *Staphylococcus aureus* and *E. coli* was 6 (16.67%) and 4 (11.1%) out of 36 fruit juices, respectively (**Table 1**).

Table 1. Bacterial pathogens detected from avocado and mango fruits juices collected from Debere Markos town

Isolates	Juice	N	Positive samples	Total	Percent	$x2$	P
Salmonella	Mango	18	0	0	0		
	Avocado	18	0				
Shigella	Mango	18	0	0	0		
	Avocado	18	0				
E. coli	Mango	18	1	4	11.1	1.125	.289
	Avocado	18	3				
S. aureus	Mango Avocado	18 18	1 5	6	16.7	3.200	.074

Previous study in Bangladesh showed that *B. cereus*, *Salmonella* species and *Staphylococcus aureus* were found in 64.91%, 7.89%, and 6.14%, of the tested samples, respectively, and (99%) the tested samples showed the presence of coliform and *E. coli* (Shakir *et al.*, 2009). Another study conducted in India documented that 27.7%, 16.6%, 38.8% of fruit juices were positive for *E. coli*, *Shigella*, and *Salmonella* spp. Positive, respectively (Lewis *et al.*, 2006).

According to the study conducted in Amravati city, India, the incidence of bacterial pathogen recorded was *E.coli* (40%), followed by *Ps. aeruginosa* (25%), *Salmonella* spp. (16%), *Proteus* spp. (9%), *S. aureus* (6%), *Klebsiella* spp. (3%), and *Enterobacter* spp. (1%) in street vended fruit juices samples (Tambekar *et al.*, 2009). Incidence of current study was differing from the study conducted in Asian countries. The probable reason for the difference may be attributed to the fruit type, geographical variation, seasonal variation, sanitation habit, and variation in methods of detection.

This study also tried to address antimicrobial susceptibility testing by means of a disc diffusion method on Mueller-Hinton Agar. The results of the antibiotic sensitivity test were interpreted, and are presented as the resistant of bacterial isolates to the antibiotics (**Table 2**).

Most isolates were susceptible to Penicillin, Ampicilin, Gentamicine, and Chloramphenicol. All isolates were resistance to Erythromycin, and most isolates were resistant to Streptomycin, Amoxicillin, and Tetracycline. The antimicrobial resistance of bacteria isolated from food and other sources, against commonly used antibiotics has increased from time to time (Vicas, 2010). According to the finding, Erythromycin was not active against all bacterial isolates. All isolates of *S.aureus* were resistant to Erythromycin and Amoxicillin. 16.7% of isolates were resistant to Tetracycline and Streptomycin, 33.3% and 66.7% to Gentamicin and Chloramphenicol, respectively. All isolates were sensitive to Penicillin and Ampicillin. High rates of drug resistance were observed for *Staphylococcus* spp. against Ampicillin (93%) and Amoxicillin (92%) (Rashed *et al*., 2013).

Table 2. Antimicrobial susceptibility patterns of pathogenic bacterial isolates from avocado and mango juice samples (in %)

Antibiotics	Bacterial isolates											
	S. aureus N = 6			*Klebsiella* spp N = 3			*E. coli* N = 4			*B. cereus* N = 2		
	R	I	S	R	I	S	R	I	S	R	I	S
Er	100	0	0	100	0	0	100	0	0	100	0	0
Amx	100	0	0	0	33.3	66.7	25	25	50	100	0	0
S	33.3	16.7	50	33.3	66.7	0	50	25	25	100	0	0
Te	16.7	0	83.3	66.7	33.3	0	75	25	0	50	50	0
C	66.7	16.7	0	0	0	100	25	0	75	50	0	50
Gm	33.3	33.3	33.3	0	0	100	25	0	75	50	0	50
P	0	0	100	66.7	33.3	0	0	0	100	0	0	100
Amp	0	16.7	83.3	66.7	0	33.3	50	25	25	50	0	50

Key: N = number of isolates, Er = Erythromycin, Amx = Amoxicillin, S = Streptomycin, Te = Tetracycline, C = Chloramphenicol, Gm = Gentamicin, P = Penicillin, Amp = Ampicillin.

Lateef (2004) reported that *S.aureus* were sensitive to erythromycin, gentamicin,and chloramphenicol and was disagreed with this finding. The works of Daniyan and Ajibo (2011), who reported that *S. aureus* was susceptible to streptomycin support this finding. Srinu *et al*., (2012) also reported that *S. aureus* was sensitive to streptomycin.

66.7% of *Klebsiella* spp. isolates were resistant to tetracycline, penicillin, and ampicillin. 33.1% of isolates were resistant to streptomycin and all were sensitive to chloramphenicol and gentamicin. Rashed *et al*. (2013) reported that *Klebsiella* spp. showed higher resistance against ampicillin (74%) and amoxicillin (72%) resistant of *Klebsiella* spp against amoxicillin was disagreed with this result. According to Stock and Wiedemann (2001), *Klebsiella* spp.were naturally sensitive or intermediate to several penicillins, all tested aminoglycosides, quinolones, tetracyclines, trimethoprim, cotrimoxazole, chloramphenicol, and nitrofurantoin.

Some *E. coli* isolates were resistant to amoxicillin, chloramphenicol, and gentamicin. 50% were resistant to streptomycin and ampicillin; all isolates were sensitive to penicillin and 75% were resistant to tetracycline. A high level of resistance was obtained among the five *E. coli* strains. Amoxicillin were not active against the strains of *E. coli* (Lateef, A., 2004). Srinu *et al*. (2012) also reported that *E. coli* was sensitive to streptomycin. Osterbald *et al*. (1999) reported that *Escherichia* spp., *Klebsiella* sp. show high sensitivity to amoxicillin and was in line with this study. Marwa *et al.* (2012) reported that most *E. coli* isolates from food were sensitive to amoxicillin but they disagreed with this result. All *B. cerues* isolates were resistant to streptomycin, erythromycin, and amoxicillin, while penicillin was active against all isolates. Agwa *et al*. (2012) found that *B. cereus* was susceptible to erythromycin and streptomycin was disagreed with this result. This result was agreed with the work of Adesetan *et al*., (2013) who reported that *B. cereus* was resistant to erythromycin.

4. CONCLUSION

The intensive and incorrect use of antimicrobial agents lead to the emergency of antimicrobial-resistant bacteria. Physicians should prescribe the correct antibiotics for the target bacteria and inform the correct use of antibiotics for patients.

Acknowledgements

I am thankful to Haramaya University School of Graduate Studies for providing me with financial support to conduct this project. I would like to thank Debre Markos University for their willingness to allow me to use the laboratory for conducting my experiments. My heartfelt thanks go to anonymous reviewers for their critical and constructive comment.

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