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Research Article

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ISOLATION, IDENTIFICATION AND ANTIMICROBIAL RESISTANCE PROFILES OF BACTERIA FROM SPOILED FRUITS IN RAWALAKOT DISTRICT POONCH, AZAD JAMMU AND KASHMIR

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ABSTRACT

Fruits play a vital role in human diet by providing essential growth factors such as vitamins and minerals in daily diet which help to live healthy life. Bacteria are important factor for spoilage of fruits. Bacterial degradation first causes softening of tissues as pectin are degraded and the whole fruit may eventually degenerate into a slimy mass. Spoilage refers to any change in fruit condition in which fruit become unacceptable or undesirable for human use. Therefore present study was conducted at Rawalakot, District Pooch AJK, to identify the bacteria (*E. coli* and *Staphylococcus aureus*) from spoiled fruit samples as well as to determine the antimicrobial resistance pattern of these isolated bacteria using antibiotic disc diffusion method. For this purpose a total of 100 spoiled fruit samples (50 samples of spoiled apple and 50 samples of spoiled banana) were randomly collected from different locations of Rawalakot including Kharick, Kasaigali, D. Chowk, Nala Bazar and Supply Bazar by random sampling technique. Results of the study showed that out of 50 samples of Banana 25(50%) samples were positive for *Staphylococcus aureus* and 25(50%) were positive for E. coli. While among 50 samples of Apple 25(50%) were positive for *E. coli* and 25(50%) were positive for *Staphylococcus aureus* respectively. For antimicrobial susceptibility test disc diffusion method was followed. *S. aureus* from apple samples the highest resistance was observed for Sulphamethox 25(100%) and Cefoxtin 25(100%) while for banana the highest %age was recorded for Amoxicillin 25(100%) and Sulphamethox 25(100%) respectively. *E. coli* from apple samples strains were highly resistant to Erythromycin 22(88%), while for banana the highest percentage was recorded for Amoxicillin 23(92%) and Erythromycin 20(80%).

Keywords: Bacteria, Antimicrobial resistance, Variance, Amoxicillin, Erythromycin

INTRODUCTION

Fruits play an indispensable part in human diet by providing essential growth and development factors (Soomar and Ranani, 2019). Fruits enclose an immense range of phytonutrients with plentiful health benefits (Jimma et al., 2022). Together with carotenoids, dietary fibers, vitamin D, amino acids, calcium and aromatic compounds avoiding heart diseases, cataracts, asthma, some forms of cancer and diabetes (Jabin et al., 2022). To reduce the risk of these diseases a balance diet of fruits and vegetable is useful (Jhee et al., 2019). Fruits having nutritional and commercial importance play an important role by providing necessary substance such as minerals and vitamins (Khatri and Sharma, 2018). As well as a font of antioxidant, crucial to defend from many infections. Resistance to first in class antibiotics such as streptomycin and penicillin discovered during the golden time of antibiotics, were observed after their initial isolation. Over the past decade consumption of fruits has been on a dominating by demand (Ashwitha et al., 2022). In current time fruits composition is increasing significantly and fruits are more treated so they are susceptible to contaminated with antibiotic resistant bacteria causing public health risk.

According to CDC worldwide, fruits are major cause of serious health problems as they are source of food borne disease in advance countries (Aliero et al., 2022). Fruits are exposed to bacterial contamination through contact with water, soil and their improper handling damaging fresh produce and rendering the product susceptible to growth of pathogenic microorganisms. Food borne illness and food borne pathogens are primarily caused by microorganisms, leading to economic costs, and morbidity (Kirk et al., 2015). In developing countries, due to unreported epidemic the statistical data on food borne disease have been increased. Consumption of microbial contaminated food and chronic sequence due to food borne infections, including immune, respiratory and cardiovascular system (Han and Bhat, 2014).

Bacterial degradation first causes softening of tissues as pectins are degraded and the full fruit can ultimately degenerate into a slender form (Rawat, 2015). The exposure and persistence of antibiotic resistance is major health challenges (Muriuki et al., 2020). Antioxidant properties, recognized for its anti-allergic, antimicrobial, antidiabetic, anti-inflammatory significance, and is a rich source of vitamin C (Jha et al., 2020). Bacteria are the major and essential factor for fruit decomposition. Bacteria are able to survive in suitable temperature with food and water resulting variation in the look, color and fragrance of the fruits (Hasan and Zulkahar, 2018).

According to FAO, a large proportion of fruits and vegetables produced worldwide is wasted annually (Jeswani et al., 2021). Spoilage of fruits and vegetables is an important food issue that has received much less research attention compared to produce related foodborne illness and processing technologies, despite its socioeconomic, food quality and food security implications (Snyder and Worobo, 2018). Although the most relevant impact of produce spoilage is socioeconomic losses, incidence of spoilage microorganisms could also be a public health threat depending on the spoilage agent (Alegbeleye et al., 2022). Fresh fruits and vegetables grown under both conventional and organic agriculture methods can be contaminated with pathogens (Maffei et al., 2016). On fresh fruits due to low pH bacteria are found in less number (Soomar and Ranani, 2019). Microbial spoilage of food is a global problem resulting in food wastage and client disappointment (Snyder and Worobo, 2018). Spoilage refers to any change in fruits condition in which fruit become unacceptable or undesirable for human use (Lalita Chaudhary and Dhaka, 2016).

Foodborne pathogens have acquired the ability to produce biofilms to survive in hostile environments (Ejaz et al., 2022). Bacteria occur within normal, sound fresh fruit tissues. They are mostly gram-negative motile rods, representatives of the Pseudomonadaceae and the Enterobacteriaceae (SAMISH et al., 1963). Microorganisms especially bacteria thrive on fresh fruits making them prone to several diseases (Jha et al., 2020). Many food pathogenic species include *Salmonella, Clostridium, Bacillus, Staphylococcus, Escherichia, Serratia* and *Listeria* posing potential risk to human health (Manzoor et al., 2016) (Sevindik and Uysal, 2021). Microbial spoilage may occur during marketing conditions, post-harvest, storage, transportation, and handling. Previously, many studies have reported the presence of bacteria including *Clostridium, Chromobacter*, *Pseudomonas*, *Xanthomonas, Bacillus, Lactobacillus, Erwinia, Flavobacterium* and *Clostridium* (Hasan and Zulkahar, 2018). Exposure to environment such as air, water and soil as well as improper manipulation can harbor a heterogeneous range of microorganisms (Sarker et al., 2018). Polygalacturonase enzyme plays role in spoilage of vegetables and. So it is necessary to produce enzymes through commercial ways so as to attain most yield in industries (Obafemi et al., 2019). Hence, observation of pathogen contamination to produce is required to build up food protection (Srisamran et al., 2022). Most of outbreak have become undetected due to lack of inadequate screening and surveillance as well as in literature very little information is available so far (Biswas et al., 2020).

The discovery of antibiotics was a defining moment in the history of mankind that revolutionized medicine and saved countless lives. Unfortunately, these "magic bullets" have been accompanied by these emerging resistant strains of pathogens. Currently, medical experts are raising real concern for a return to the pre antibiotic age (Aslam et al., 2018). Many decades later, the first patients were treated with antibiotics. Bacterial infections are once again a threat (Spellberg and Gilbert, 2014). Antibiotic resistance is a serious universal problem to human health, causing hundreds of thousands of fatalities each year (Pehrsson et al., 2016). Resistance genes can associate in clusters and be transferred together as well (Schaack et al., 2010). Evolution of bacterial resistance to antibiotics is therefore a natural process and would exist even absent human mismanagement (Fair and Tor, 2014). Antimicrobial Resistance (AMR) is a growing problem in the 21st century and one of the most important jeopardies to universal public health World Health (Organization, 2000).

MATERIALS AND METHODS

Study Design: It was a quantitative cross-sectional investigation to find out the antimicrobial susceptibility pattern of *Escherichia coli* and *Staphylococcus aureus*. The study was conducted to isolate and characterize the resistance profiles of bacteria from spoiled fruits in Rawalakot District Poonch, AJK.

Sampling: Fruits market in Rawalakot Kharick, Kasaigali, Nala Bazar, Supply Bazar and D-Chowk were selected for sample collection in Rawalakot district Poonch, AJK. Total of 100 samples of spoiled fruits (50 samples of spoiled apple and 50 sample of spoiled banana) was collected by random sampling. For the spoilage symptoms like odour, colour, consistency and many other types of visible symptoms, all type of spoiled fruit sample was examined attentively. For the purpose of isolating bacteria one gram of spoiled fruit apple and banana was homogenized in 100 ml of peptone water and incubated at 37°C overnight.

Media and Growth Condition: Selective media, including Nutrient Agar, Nutrient Broth, MacConkey Agar, and Blood Agar, were used for the isolation of

Escherichia coli and *Staphylococcus aureus*. Sterilization of culture media, Petri plates, wire loops, flasks, and test tubes was performed using an autoclave. To ensure sterility and check for contamination, all media were incubated at 37°C.

Isolation of *E. coli* and *S. aureus:* The medium were prepared by taking the required amount of media weighed by digital measuring balance and dissolve in sterile water. Mixture was well mixed by shaking. Prepapred selective medium were autoclaved and then pour media in petri plates. Then petri plates were placed in incubator for 24 hours at 37°C to check sterility. Each sample was streaked onto a newly made selective medium in a volume in 100ml. The plates were incubated at 37°C for 24 hours. *E. coli* produce pink color colonies on MacConkey agar while *S. aureus* produces white color colonies on Blood agar.

Identification of *E. coli* and *S. aureus:* *S. aureus* and *E. coli* were identified on the basis of cultural and morphological characters. For cultural media selective media were used. MacConkey agar was used for *E. coli* and Blood agar was used for *S. aureus.* For conventional method gram staining was used.

Disc Diffusion Method: Kirbay-Bauer to check the susceptibility of isolated bacteria against antibiotics disc diffusion method was used. The zones of inhibition obtain each bacterium after incubation with the antibiotic disc was compared following CLSI standard (Kousar et al., 2021)**.** The pure cultures of bacteria was sub-cultured on nutrient agar medium. Each strain was swabbed selected uniformly onto individual plate using sterile cotton swabs. Selective antibiotics disc was placed on the inoculated agar (CLSI 2012). After incubation at 37 C for 24 hours, the different level of zones of inhibition of bacteria was measured in mm.

Statistical Analysis: All experiment was performed in triplicate and data was expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

Isolation and Identification of *E. coli* and *Staphylococcus aureus* : The present study was conducted in tehsil Rawalakot of District Pooch AJK, to identify bacteria (*Escherichia. coli* and *Staphylococcus aureus*) from spoiled fruit samples as well as to determine the antimicrobial resistance pattern of these isolated bacteria using antibiotic disc diffusion method. For this purpose a total of 100 spoiled fruit samples containing 50 Banana samples as well as 50 of Apple were collected from different locations of Rawalakot including Kharick, Kasaigali, D. Chowk, Nala bazar and Supply Bazar by random sampling technique (Figure 1). Using selective media like Blood agar used for isolation of *S. aureus* produced white color colonies as shown in (Figure 2). While MacConkey agar was used for isolation of Escherichia coli produced pink color colonies respectively shown in (Figure 3).

Out of 50 samples of Banana 25(50%) samples were positive for *Staphylococcus aureus* and 25(50%) were positive for *E. coli*. While among 50 samples of Apple 25(50%) were positive for *E. coli* and 25(50%) were positive for *Staphylococcus aureus* respectively as shown in (Table 1). From each sample 3 isolates were obtained and a total of 150 isolates of *Staphylococcus aureus* as well as 150 of *E. coli* were selected in addition by sequential quadrant streaking were obtained on pure culture. A total 150 *Staphylococcus* isolates 75 were from Apple samples (Table 1) and 75 were collected from banana samples shown in (Table 2). Similarly in case of *E. coli* 75 isolates were selected from apple samples (Table 3) and 75 from banana samples (Table 4) respectively and all were found positive for pathogens.

Figure 1: Showing spoiled fruit samples for bacterial screening.

Figure 2: *S. aureus* colonies on selective media (Blood agar)

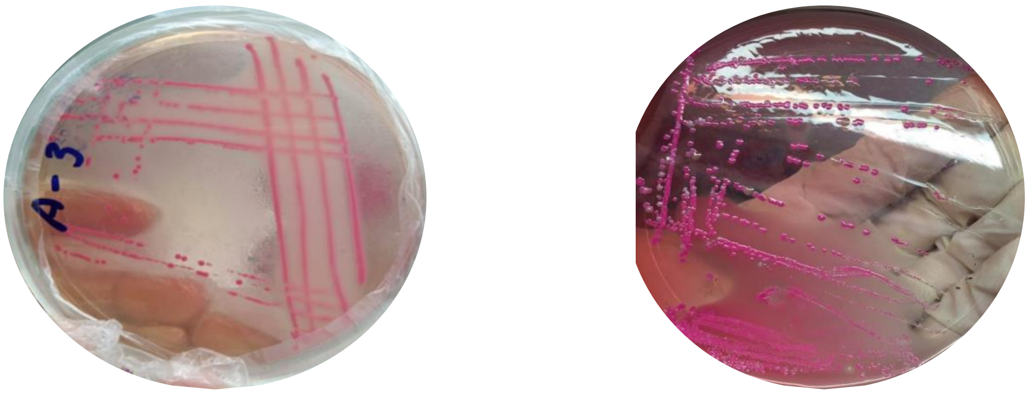


Figure 3: *E. coli* colonies on selective media (MacConkey agar).

Table 1: Frequency distribution of *Staphylococcus aureus* in Apple.

Type of Sample	Source	No. of tested samples (Apple)	No. of positive samples for *S. aureus*	No. of isolates	%age
Apple	Ksaigali	5	5	15	25%
	Kharick	5	5	15	25%
	Nala Bazar	5	5	15	25%
	D. chowk	5	5	15	25%
	Supply Bazar	5	5	15	25%
	Total	25	25	75	25%

Table 2: Frequency distribution of *Staphylococcus aureus* in Banana

Type of Sample	Source	No. of tested samples (Banana)	No. of positive samples for *S. aureus*	No. of isolates	%age
Banana	Ksaigali	5	5	15	25%
	Kharick	5	5	15	25%
	Nala Bazar	5	5	15	25%
	D. chowk	5	5	15	25%
	Supply Bazar	5	5	15	25%
	Total	25	25	75	25%

Table 3: Frequency distribution of *Escherichia coli* in Apple

Type of Sample	Source	No. of tested samples (Apple)	No. of positive samples for *E. coli*	No. of isolates	%age
Apple	Ksaigali	5	5	15	25%
	Kharick	5	5	15	25%
	Nala Bazar	5	5	15	25%
	D. chowk	5	5	15	25%
	Supply Bazar	5	5	15	25%
	Total	25	25	75	25%

Table 4: Frequency distribution of *E.coli* in Banana

Type of Sample	Source	No. of tested samples (Banana)	No. of positive samples for *E. coli*	No. of isolates	%age
Banana	Ksaigali	5	5	15	25%
	Kharick	5	5	15	25%
	Nala Bazar	5	5	15	25%
	D. chowk	5	5	15	25%
	Supply Bazar	5	5	15	25%
	Total	25	25	75	25%

Phenotypic Characterization: For further conformation isolated *Escherichia coli* and *Staphylococcus aureus* were consequently analyzed for morphological and cultural characteristics. The 75 isolates of *S. aureus* and 75 isolates of *E. coli* were maintained by sub culture over nutrient agar. In addition optical microscope was usedto investigate bacterial cell morphology.

Identification of *S. aureus:* For cultural identification of *S. aureus* isolates was sub-culture on selective media Blood agar produced white color colonies. For conventional identification the microscopic examination of Grams stained smear of *S. aureus* from blood agar seen in (Figure 4) showed characteristics of Gram positive, purple colored, spherical to ovoid organisms arranged in pairs, chains or in clusters showed growth at 37˚C shown in (Table 5). These two results were confirmed the identification of isolates as belonging to *S. aureus.*

Identification of *E. coli:* For cultural identification of *E. coli* isolates was sub-culture on selective media MacConkey agar producing pink color colonies. For conventional identification the microscopic examination of Grams stained smear of *E. coli* from MacConkey agar seen in (Figure 5) showed characteristics of Gram negative, dark pink colored, small rod shape organisms arranged in single, in pair or in short chain showed growth at 37˚C shown in (Table 6). These two results were confirmed the identification of isolates as belonging to *E. coli.*

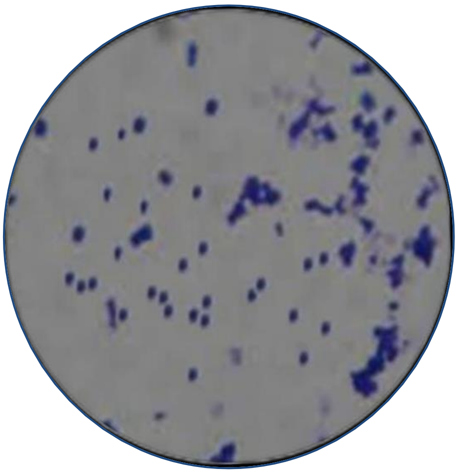


Figure 4: Gram staining *S. aureus* showed purple stains.

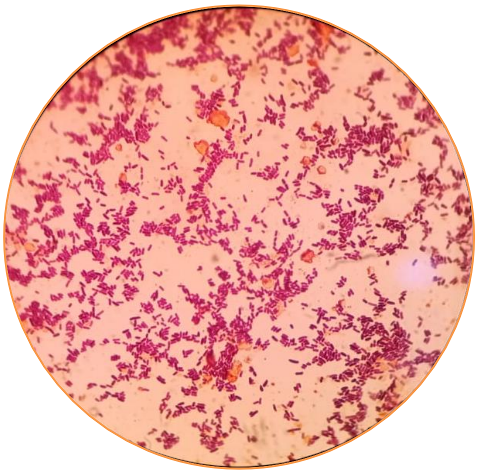


Figure 5: Gram staining *E. coli* showed dark pink stain.

Table 5: Phenotypic characteristics of *Staphylococcus aureus*

Test (Characteristics)	Reaction
Morphological parameters	
Gram reaction	+ive
Shape	Spherical to ovoid (Cocci)
Color	Purple
Arrangement	Pairs, chains or in clusters
Cultural Parameters	
Blood agar	Round smooth colonies of white color
Growth at 37˚C	+ive

Table 6: Phenotypic characteristics of *E. coli.*

Test (Characteristics)	Reaction
Morphological parameters	
Gram reaction	–ive
Shape	Rod shape (Bacilli)
Color	Dark pink
Arrangement	Single or in Pairs
Cultural Parameters	
MacConkey agar	Pink small color
Growth at 37˚C	–ive

Antimicrobial Susceptibility Test: For antimicrobial susceptibility test disc diffusion method was followed. Well isolated colony of *E. coli* from MacConkey agar plate and *S. aureus* from Blood agar media were picked with sterilized wire loop. Suspend the colony material properly in nutrient broth and move the loop slightly forward and backward to get a homogenious suspension. Put them in incubator for 24 hours to get proper growth of *Escherichia coli and Staphylococcus aureus*. After *S. aureus* and *E. coli* growth, a bacterial lawn was manually created by equally distributing a large number of bacteria on agar plate using a sterile cotton swab. A sterile cotton swab was dipped into the inoculum and pressed against the tube to remove any excess medium. To create a bacterial lawn, swab the plates surface area completely. The agar plate was streaked with a swab in one direction, rotated once by 120 degrees, streaked again, and so on to achieve uniform growth. Disc diffusion method was used to check the susceptibility of isolated bacteria against antibiotics. The plate was then covered with antibiotic discs. Each disc was firmly pressed onto the agar with the use of sterilized forceps to secure its attachment. The plates were incubated overnight, usually at 37°C. After 24 hours of incubation, use a metric ruler to determine the diameter of disc and zones of inhibition. Seven different antibiotics including Azithromycin (15µg), Amoxicillin (30µg), Sulphamethox (25µg), Streptomycin (10µg), Tetracycline (30µg), Erythromycin (15µg) and Cefoxtin (30µg) were used for disc diffusion method. Compared the outcomes to the CLSI. The outcomes were classified as Susceptible (S), Intermediate (I) and Resistance (R). The concentration and interpretative standard zone diameter of different antimicrobial agent was observed as shown in (Table 7).

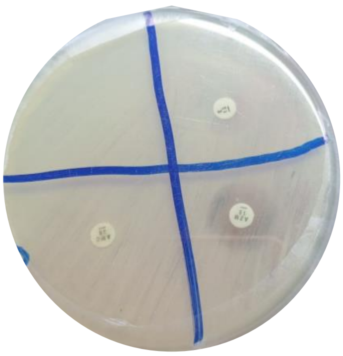
Table 7: Concentration and interpretative standard zone diameter of different antimicrobial agents.

Antimicrobial Disk	Content µg	Resistance	Intermediate	Susceptibility
Azithromycin	15 µg	≤13	14-17	≥18
Amoxicillin	30 µg	≤ 13	14-16	≥17
Sulphamethox	25 µg	≤ 10	11-15	≥16
Streptomycin	10 µg	≤ 11	12-14	≥15
Tetracycline	30 µg	≤ 14	15-18	≥19
Erythromycin	15 µg	≤ 15	16-20	≥ 21
Cefoxtin	30 µg	≤15	16-20	≥ 21

Antimicrobial susceptibility test of bacteria isolates (*E. coli* and *S. aureus*) with disc diffusion method in accordance to CLSI 2012 standard. Resistance: Intermediate and Susceptibility.

Antimicrobial Susceptibility Patterns of *S. aureus* From Spoiled Apple: Out of 75 isolates from apples the highest resistance was observed for Sulphamethox 25(100%) and Cefoxtin 25(100%) while lowest resistance was shown in Streptomycin 6(24%) respectively. The resistance patterns against other antibiotics were observed as Azithromycin 7(28%), Amoxicillin 21(84%), Erythromycin 8(32%) and Tetracycline 20(80%) respectively. Most *S. aureus* were intermediate to Tetracycline 3(12%) followed by Amoxicillin 3(12%) followed by Erythromycin 1(4%) followed by Azithromycin 0(0%) followed by Sulphamethox 0(0%) while lowest intermediate were followed by Streptomycin 0(0%) and followed by Cefoxtin 0(0%). Most of *S. aureus* were susceptible to Streptomycin 19(76%) followed by Azithromycin 18(72%) followed by Erythromycin 16(64%) followed by Tetracycline 2(8%) followed by Amoxicillin 1(4%) while lowest susceptible were followed by Sulphamethox 0(0%) and followed by Cefoxtin 0(0%) respectively shown in (Table 8 and Figure 6 and 7).

Antimicrobial Susceptibility Patterns of *S. aureus* From Spoiled Banana: In the case of banana total 75 isolates from banana the highest resistance percentage were recorded for Amoxicillin 25(100%) and Sulphamethox 25(100%) while lowest resistance was shown in Streptomycin 0(0%) correspondingly. The resistance patterns against other antibiotics were observed as Azithromycin 5(20%), Cefoxtin 22(88%), Erythromycin 9(36%) and Tetracycline 16(64%). Most of *S. aureus* were intermediate to Azithromycin 5(20%) followed by Tetracycline 5(20%) followed by Cefoxtin 3(12%) followed by Erythromycin 2(8%) while lowest intermediate were followed by Amoxicillin 0(0%) Sulphamethox 0(0%) and followed by Streptomycin 0(0%). Most of *S. aureus* were susceptible to Streptomycin 25(100%) followed by Azithromycin 15(60%) followed by Erythromycin 14(56%) followed by Tetracycline 4(16%) while lowest susceptible were followed by Amoxicillin 0(0%) Sulphamethox 0(0%) and followed by Cefoxtin 0(0%) respectively shown in (Table 9 and Figure 8 and 9).



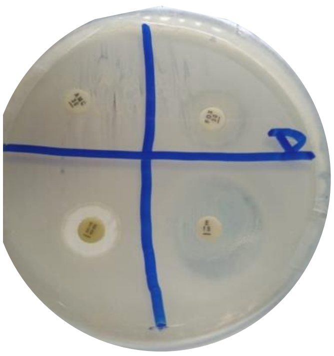


Figure 6: Zone of inhibition of *S. aureus* from spoiled apple

Table 8: Overall percentage of antibiotic resistance pattern of *S. aureus* isolated from spoiled apple.

Antibiotics	Resistance	Intermediate	Susceptibility	Mean ± S.D
Azithromycin (15 µg) n =75	7(28%)	0(0%)	18(72%)	22.2 ± 14.9638
Amoxicillin (30 µg) n =75	21(80%)	3(12%)	1(4%)	4.52 ± 6.4557
Sulphamethox (25 µg) n =75	25(100%)	0(0%)	0(0%)	0 ± 0
Streptomycin (10 µg) n =75	6(24%)	0(0%)	19(76%)	20.8 ± 12.7932
Tetracycline (30 µg) n =75	20(80%)	3(12%)	2(8%)	6.64 ± 7.5546
Erythromycin (15 µg) n =75	8(23%)	1(4%)	16(64%)	24.88 ± 18.1138
Cefoxtin (30 µg) n =75	25(100%)	0(0%)	0(0%)	3.16 ± 5.0228836

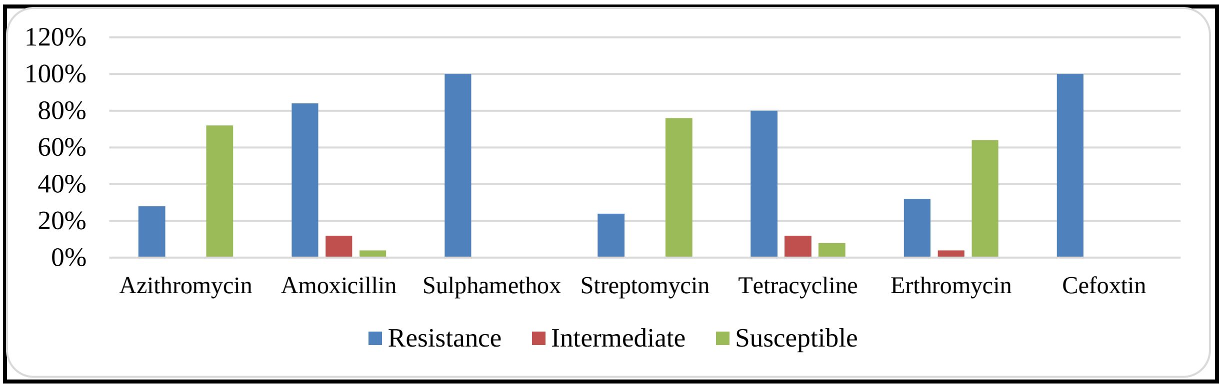


Figure 7**:** Antibiotic resistance pattern of *S. aureus* from spoiled apple

Table 9: Overall percentage of antibiotic resistance pattern of *Staphylococcus aureus* isolated from spoiled banana.

Antibiotics	Resistance	Intermediate	Susceptibility	Mean ± S.D
Azithromycin (15 µg) n =75	5(20%)	5(20%)	15(60%)	19.84 ± 11.6357
Amoxicillin (30 µg) n =75	25(100%)	0(0%)	0(0%)	1.32 ± 3.14536
Sulphamethox (25 µg) n =75	25(100%)	0(0%)	0(0%)	0 ± 0
Streptomycin (10 µg) n =75	0(0%)	0(0%)	25(100%)	25.12 ± 4.2848
Tetracycline (30 µg) n =75	16(64%)	5(20%)	4(16%)	13.56 ± 3.8607
Erythromycin (15 µg) n =75	9(36%)	2(8%)	14(56%)	17.88 ± 13.5225
Cefoxtin (30 µg) n =75	22(88%)	3(12%)	0(0%)	7.6 ± 7.6130003

Figure 8: Zone of inhibition of *S. aureus* from spoiled banana.

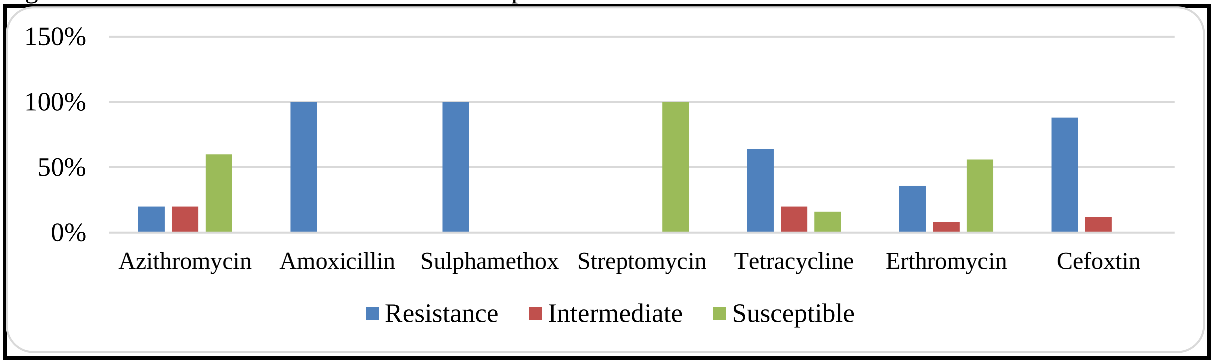


Figure 9: Antibiotic resistance pattern of *S. aureus* from spoiled banana.

Antimicrobial Susceptibility Patterns of *E. coli* From Spoiled Apple: To check the susceptibility of isolated bacteria against antibiotics disc diffusion method was used. For this test total 150 isolates of *E. coli* 75 from apples and 75 from bananas were used. Together seven different antibiotics including Azithromycin, Amoxicillin, Sulphamethox, Streptomycin, Tetracycline, Erythromycin and Cefoxtin were also used for disc diffusion method.

Out of 75 isolates from apples the highest resistance was observed for Erythromycin 22(88%) while lowest resistance was shown in Sulphamethox 8(32%) respectively. The resistance patterns against other antibiotics were observed as Azithromycin 12(48%), Amoxicillin 19(76%), Streptomycin 11(44%), Cefoxtin 13(52%) and Tetracycline 14(56%) respectively. Most of *E. coli* were intermediate to Cefoxtin 8(32%) followed by Azithromycin 4(16%) followed by Streptomycin 3(12%) followed by Tetracycline 3(12%) followed by Amoxicillin 3(12%) while the lowest intermediate were followed by Erythromycin 2(8%) and followed by Sulphamethox

2(8%). Most *E. coli* were susceptible to Sulphamethox 15(60%) followed by Streptomycin 11(44%) followed by Azithromycin 9(36%) followed by Tetracycline 8(32%) followed by Cefoxtin 4(16%) followed by Amoxicillin 3(12%) while lowest susceptible was followed by Erythromycin 2(8%) shown in (Table 4.10 and Figure 4.10, 4.11).

Table 10: Overall percentage of antibiotic resistance pattern of *Escherichia coli* isolated from spoiled Apple

Antibiotics	Resistance	Intermediate	Susceptibility	Mean ± S.D
Azithromycin (15 µg) n =75	12 (48%)	4(16%)	9(36%)	12.28 ± 9.235
Amoxicillin (30 µg) n =75	19(76%)	3(12%)	3(12%)	5.12 ± 7.8438
Sulphamethox (25 µg) n =75	8(32%)	2(8%)	15(60%)	14.2 ±10.583
Streptomycin (10 µg) n =75	11(44%)	3(12%)	11(44%)	11.4 ± 8.8354
Tetracycline (30 µg) n =75	14(56%)	3(12%)	8(32%)	11.8 ± 10.033
Erythromycin (15 µg) n =75	22(88%)	2(8%)	1(4%)	5.04 ± 7.7431
Cefoxtin (30 µg) n =75	13(52%)	8(32%)	4(16%)	12.88 ± 8.176

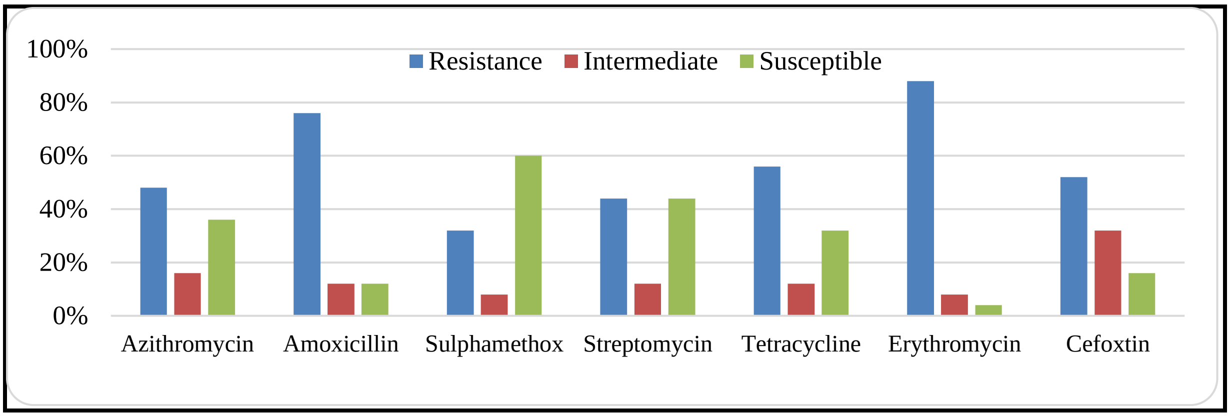


Figure 10. Zone of inhibition of *E. coli* from spoiled apple.

Figure 11. Antibiotic resistance pattern of *E. coli* from spoiled apple.

Antimicrobial Susceptibility Patterns of *E. coli* from Spoiled Banana: In the case of banana of total 75 isolates the highest %age was recorded for Amoxicillin 23(92%) while lowest resistance was shown in Streptomycin 9(36%) respectively. The resistance patterns against other antibiotics were observed as Azithromycin 10(40%), Cefoxtin 17(68%), Sulphamethox 10(40%), Erythromycin 20(80%) and Tetracycline 12(48%). Most of *E. coli* were intermediate to Cefoxtin 8(32%) followed by Sulphamethox 7(28%) followed by Azithromycin 5(20%) followed by Tetracycline 3(12%) followed by Amoxicillin 2(8%) followed by Streptomycin 2(8%) while lowest intermediate was followed by Erythromycin 1(4%). Most of *E. coli* were susceptible to Streptomycin 14(56%) followed by Azithromycin 10(40%) followed by Tetracycline 10(40%) followed by Sulphamethox 8(32%) followed by Erythromycin 4(16%) while lowest susceptible were followed by Cefoxtin 0(0%) and followed by Amoxicillin 0(0%) shown in (Table 4.11 and Figure 12 and 13).

Table 11: Overall percentage of antibiotic resistance pattern of *Escherichia coli* isolated from spoiled Banana

Antibiotic discs	Resistance	Intermediate	Susceptibility	Mean ± S.D
Azithromycin (15 µg) n =75	10(40%)	5(20%)	10(40%)	13.56 ± 8.3719
Amoxicillin (30 µg) n =75	23(92%)	2(8%)	0(0%)	4.4 ± 6.05530

Sulphamethox (25 µg) n =75	10(40%)	7(28%)	8(32%)	10.28 ± 9.1720
Streptomycin (10 µg) n =75	9(36%)	2(8%)	14(56%)	12.56 ± 7.8479
Tetracycline (30 µg) n =75	12(48%)	3(12%)	10(40%)	16.64 ± 10.4637
Erythromycin (15 µg) n =75	20(80%)	1(14%)	4(16%)	8.28 ± 9.3270
Cefoxtin (15 µg) n =75	17(68%)	8(32%)	0(0%)	8.64 ± 8.0668

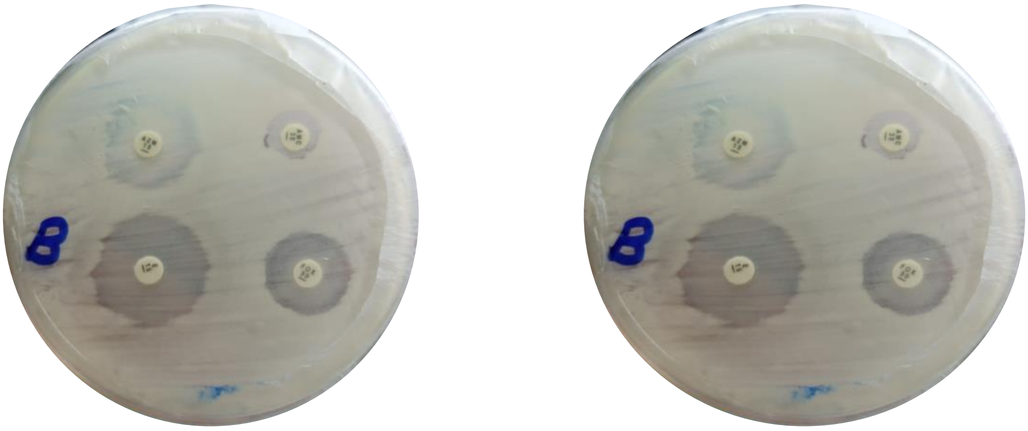


Figure 12: Zone of inhibition of *E. coli* from spoiled banana.

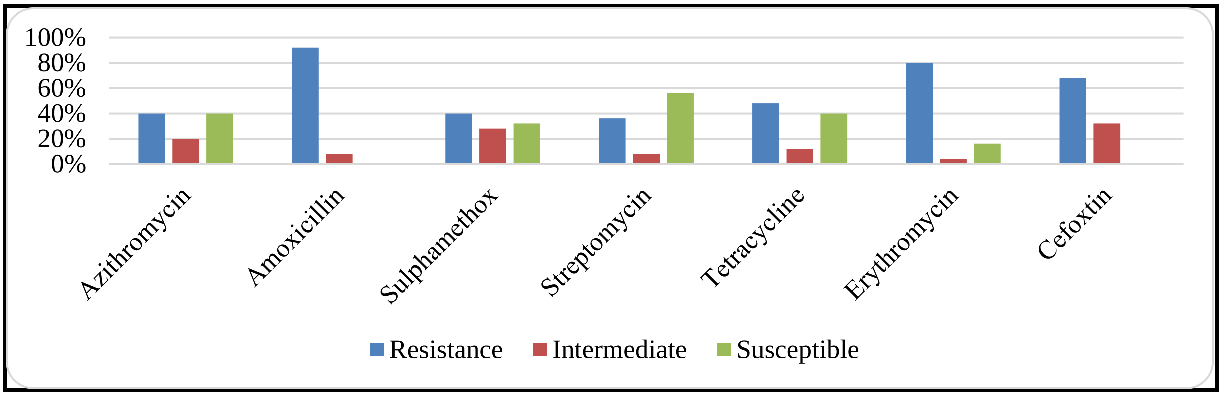


Figure 13: Antibiotic resistance pattern of *E. coli* from spoiled banana.

Since the discovery of antibiotic agents more than ten years ago. Antibiotics have prevented countless deaths from infectious diseases and revolutionized contemporary medical operations like organ transplant, cancer therapy and surgery. A slow but persistent rise in antibiotic resistance, which occurs when bacteria make antibiotics infective, poses a threat to erase these significant advancements and return the world to its pre-antibiotic state. While increase use of antibiotics inhuman health sector has played a significant role in the greater usage of these wonder medications in food animal production have come to light in recent year. A significant public health issue is rise of germs that are resistant to antibiotics. The rapid urbanization, congestion and inadequate sensation system in Rawalakot considerably accelerate the spread of numerous diseases. The increase in the infection is to blame for the rising use and occasionally abuse of antibiotics. In this work we identified the pattern of antimicrobial resistance in subset of *S. aureus* and *E. coli* isolated from spoiled apple and banana in Rawalakot. For the purpose of investigation, about 100 sample of spoiled fruits were collected. 50 sample of spoiled apple and 50 sample of spoiled banana were collected from different fruit markets. Test on sample of banana and apple showed apple and banana contain the bacteria that are resistant to antibiotics. While 50 samples out of 25 spoiled apple and 25 spoiled banana were chosen for *E. coli*. While 50 samples out of 25 spoiled apple and 25 spoiled banana were chosen for *S. aureus*. In this study, *E. coli* and *S. aureus* are tested for antibiotics sensitivity using seven regularly used antibiotics including Tetracycline, Amoxicillin, Azithromycin, Streptomycin, Sulphamethox, Cefoxtin and Streptomycin.

According to this study 100% of banana and apple had isolated *E. coli*. The 100% of *E. coli* isolation was closely comparable to a study completed in Kalaburagi, where the *E. coli* isolation was 86.666% (Gundappa et al., 2024). A study conducted in India, spoiled apple and banana had isolation rate of *E. coli* (22.32%) and *S. aureus* (22.76%) respectively (Hamalata and Virupakshaiah, 2016). Compared to this study this *E. coli* and *S. aureus* isolation rate was low. From India another study was reported 5 isolates identified to *E. coli* and *S. aureus* spp were subjected to antibiotics resistance test and study revealed that *Staphylococcus spp.* were resistant to Ofloxacin, Ampicillin, whereas *E. coli* showed resistance toward Ciprofloxacin, Gentamycin, Ampacillin, Chloramphenol respectively (Jha et al., 2020). A similar study was reported from Lahore to isolate the bacterial species from spoiled fruits. Resistance was shown by all the bacterial species against penicillin but the level of resistance or sensitivity varied among all the strains and 15/19 were sensitive to Erythromycin, Streptomycin, Cefriaxone concentration respectively (Roheen and Latif, 2021). Both of these reports used different antibiotics as compared to this study but this show that most of bacterial species showed resistance against many antibiotics. Present study found that 50% of spoiled apple samples tested positive for *E. coli* and 50% samples of spoiled banana samples tested positive for *S. aureus*. From Bangladesh another study was reported fifty four rotten sample of fruits were examined of which 35 species of bacteria were isolated. These bacteria were identified as *Staphylococcus aureus, Salmonella,* and *Escherichia coli*. *S. aureus* (28%) and *E. coli* (20%) was most predominant. *E. coli* and *S. aureus* was resistant to Erythromycin and Amoxicillin (Aboney *et al.,* 2020). (Islam et al., 2010) from Bangladesh *E. coli* were isolated from spoiled apple. The spoiled apple had isolation rate of *E. coli* 18.52%.

Staphylococcus aureus are gram positive aerobic microorganisms responsible for skin infection, osteomyelitis, pneumonia and endocarditis so it cause mortality and morbidity in humans. On the other hand E. coli are gram negative bacteria rod shaped organisms usually its strains are harmless but can be stereotype as may be the cause of serious food poisoning in individuals (Cole et al., 2018). Total of 100 spoiled fruit samples containing 50 Banana samples as well as 50 of Apple were collected from different locations of Rawalakot including Kharick, Kasaigali, D. Chowk, Nala bazar and Supply Bazar by random sampling technique. Using selective media like Blood agar used for isolation of S. aureus produced white color colonies. While MacConkey agar was used for isolation of *Escherichia coli* produced pink color colonie.

Out of 50 samples of Banana 25(50%) samples were positive for *Staphylococcus aureus* and 25(50%) were positive for *E. coli*. While among 50 samples of Apple 25(50%) were positive for *E. coli* and 25(50%) were positive for *Staphylococcus aureus* respectively. From each sample 3 isolates were obtained and a total of 150 isolates of *Staphylococcus aureus* as well as 150 of *E. coli* were selected in addition by sequential quadrant streaking were obtained on pure culture. A total 150 *Staphylococcus* isolates 75 were from Apple samples and 75 were collected from banana samples. Similarly in case of *E. coli* 75 isolates were selected from apple samples and 75 from banana samples respectively and all were found positive for pathogens. Out of 75 isolates of *E. coli* from apple the highest resistance was observed for Erythromycin 22(88%) while lowest resistance was observed in Sulphamethox 8(32%) respectively. The resistance patterns against other antibiotics were observed as Azithromycin 12(48%), Amoxicillin 19(76%), Streptomycin 11(44%), Cefoxtin 13(52%) and Tetracyline 14(56%). In case of banana total 75 isolates the highest %age was recorded for Amoxicillin 23(92%), while lowest resistance was shown in Streptomycin 9(36%). The resistance patterns against other antibiotics were observed as Azithromycin 10(40%), Amoxicillin 19(76%), Streptomycin 11(44%), Sulphamethox 10(40%), Cefoxtin 17(68%) and Tetracyline 12(48%). Out of 75 isolates from apple the highest resistance was observed for Erythromycin 22(88%) while lowest resistance was observed in Sulphamethox 8(32%) respectively.

S. aureus isolates from spoiled apple the highest resistance were observed for Sulphamethox 25(100%), and Cefoxtin 25(100%) while lowest resistance was observed in Streptomycin 6(24%), respectively. The resistance patterns against other antibiotics were observed as Azithromycin 7(28%), Amoxicillin 21(84%), Erythromycin 8(32%), and Tetracyline 20(80%). In case of banana total 75 isolates the highest %age was recorded for Amoxicillin 25(100%), while lowest resistance was shown in Streptomycin 0(0%). The resistance patterns against other antibiotics were observed as Azithromycin 5(20%), Erythromycin 9(36%), Sulphamethox 25(100%), Cefoxtin 22(88%) and Tetracyline 16(64%).

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