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Original Article

Antibiotic Efficacy of Commercially Available Antibiotics on Indigenous Microbes Isolated from Rotten Fruits

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ABSTRACT

The human commonly consume fruits as food. Indigenous microbes are responsible for the spoilage of fruits. If bacteria spoil consumed fruit, they can cause infections in the human body that may lead to severe consequences. Some commercially available drugs are effective against an infection, and some show no or little effect. **Objective:** To check the efficacy of locally available drugs against indigenous microbes that are commonly responsible for infections in natives. **Methods:** Microbes isolated from rotten fruits are used to check that the antibiotics available in our market are effective against them. Different antibiotics are used against these bacteria. Control antibiotics determine the efficacy of each antibiotic. **Results:** All the antibiotics are effective but to a different extent. The presence of the active compound in the drug determines its effectiveness. If the active compound is present in pure form and adequate amounts in the dosage of the drug, it will be effective. Bacteria become resistant to the drugs, which is the major issue faced. **Conclusions:** Antibiotic resistance and specificity of drugs are also important factors that determine the efficacy of the drugs. Antibiotic resistance is influenced by the uncontrolled, unnecessary, and recommended use of antibiotics in society. The specificity of the drug to the microbes also contributes to the effectiveness of antibiotics.

INTRODUCTION

The bacteria present in an environment are responsible for the many infectious diseases in human being as well as in other organisms. Human can be infected by these microbes when came into contact with contaminated surfaces or when consume the spoiled food [1]. Due to this interaction bacteria can cause many symptoms of illness in human body. The antibiotics available in commercial market are commonly used by the natives [2]. These antibiotics should be effective against the indigenous microbes because they are the major cause of infections and disease. Antibiotics used for treatment bacterial infections kill bacteria (bactericidal) and inhibit their growth (bacteriostatic). they can induce drug resistance in these microbes too. Serious adverse consequences can be caused by excessive and long-term use of antibiotics [3]. Fruits offer a conducive habitat for the proliferation and development of various microorganisms, particularly bacteria [4]. The tissues within fruits contain a significant concentration of diverse carbohydrates, minerals, vitamins, and amino acids [5]. The second point is as

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follows. Spoilage is a term used to describe any alteration in the state of food that renders it unattractive or unsuitable for human consumption [6]. Bacterial organisms the initial stage of spoilage involves the weakening of tissues due to the degradation of pectins, ultimately leading to the complete degeneration of the fruit into a slimy mass. The subsequent metabolic processes involve the breakdown of starch and sugars, resulting in the emergence of undesirable odors and smells, as well as the production of lactic acid and ethanol [7]. The proliferation of undesired contaminating bacteria not only leads to the degradation of sensory and organoleptic attributes of food, but also poses a risk of causing illnesses [8]. The majority of pathogenic bacteria included in food products originate from the intestinal tract, while a subset can also be detected in the nasal passages, throat, hair, and skin. Therefore, it is frequently observed that food handlers serve as a primary conduit for both contamination and cross-contamination. Foodborne illnesses caused by foodborne microorganisms represent a significant public health concern that is closely linked to food hygiene practices and the broader concept of food safety [9]. Foodborne infections have garnered considerable public health scrutiny in the last five decades, as the recognition of microbial contamination as a substantial global issue has grown [10]. These ailments provide a significant challenge in both developed and developing nations. Diseases transmitted via the ingestion of food or water that has been polluted, particularly in regions with inadequate sanitation, encompass hepatitis A, hepatitis E, typhoid fever, as well as diarrhea and dysentery [11]. The advent of antibiotics brought about a significant transformation in the approach to treating infectious diseases. The precise anticipation of antibiotic effectiveness is crucial for the effective management of bacterial infections [12]. In addition to genetically encoded pathways of antibiotic resistance, there is a limited understanding of the factors that determine antibiotic susceptibility during infection, leading to frequent instances of treatment failure. Conventional antibiotic susceptibility testing disregards the influence of external factors on antibiotic susceptibility within the intricate infection milieu, thereby rendering it an inadequate indicator of antibiotic treatment efficacy. The excessive and inappropriate use of antibiotics is resulting in the development of resistance towards these crucial pharmaceutical agents [13]. The emergence of resistance to several antimicrobial drugs is being observed in bacterial infections on a global scale. Therefore, it is crucial for clinical microbiologists to furnish doctors with precise information necessary for the selection of antibiotics for patient therapy and care [14]. The antibiotics available in market must be effectual. There is a need of proper inspection of antibiotics that are available in local commercial market. The recommendation of antibiotic and their dosage must according to the suitability against agent and severity of the infection [15]. Use of unproductive drugs may lead to the severe consequences and can increase the risk of fatalness of disease. Most suitable and effective antibiotics should be used against the bacterial infections [16]. The future of antibiotics needs upheaval in a field that stood on highly traditional methods of development and discovery. This will need considerable changes in policies, a numeric understanding of the societal value of these medicines, and financing in alternatives to conventional drugs, including monoclonal antibodies, narrow-spectrum drugs, vaccines, and bacteriophage coupled with highly efficient diagnostics [17]. The objectives of this study were to isolate the microbes responsible for the Spoilage of fruits and to analyse the antibiotic activity of commercially available antibiotics against the indigenous bacteria.

METHODS

Samples of rotten fruits are collected from the local fruit market of Mirpur. Two types of unwashed and unprocessed spoiled fruits comprising of viz. banana and peach were collected in plastic zip bags [18]. These samples are brought in to the laboratory of the Department of Biotechnology Mirpur University of Science and Technology (MUST), for further analysis. Microbes are isolated from the spoiled site of the fruits; suspensions of rotten parts are made and then serially diluted. These serial dilutions spread over the media plates and incubated. Bacterial colonies grow on the media that are identified and Isolated. These colonies are used to form pure culture and inoculum [19]. Rotten parts of spoiled fruits were cut by using sterile knife and weigh by using digital weighing balance. 1g of each sample was used. To prepare the suspension autoclaved distilled water and sterile test-tubes. 1g pieces of rotten fruits added to the 9ml of autoclaved water and the test tubes were shacked to form a homogenized mixture. In this way the 10ml suspensions of fruits components in water, formed. As the suspension formed from the spoiled part of fruits so it contains the huge number of microbes in it. The suspensions formed from the rotten fruits may have a high microbe's density. To get the isolated colonies of bacteria serial dilutions were made. The dilutions were made by adding 1ml of previous dilution, up to 10-6 [2(6)=12] as shown in Table 1.

Table 1: Serials Dilutions of Samples

Dilutions	Initial Volume (mL)	Volume added From Previous Dilution (mL)	Final Volume (mL)
10-1	09	1g (weight) Fruit Sample	10
10-2	09	01	10
10-3	09	01	10

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10-4	09	01	10
10-5	09	01	10
10-6	09	01	10

All the components of agar media e.g., Yeast Extract, Peptone, Sodium Chloride & agar are added in distilled water in measured quantity according to standard shake to mix the components in water. Composition of nutrient agar is shown in Table 2.

Table 2: Ingredients of Agar Media

Yeast Extract	3.0	1.5
Peptone	5.0	2.5
Sodium Chloride	8.0	4.0
Agar	15.0	7.5

Table 3: Growths on Plates

Plate #	Dilutions	Growths
1.	10-1	Very Dense
2.	10-2	Dense
3.	10-3	Dense
4.	10-4	Less Dense
5.	10-5	Slightly Visible
6.	10-6	Not Visible

Plates of 10^{-4}, 10^{-5}, 10^{-6} dilutions are allowed to incubate for next 24 hours After the next 24 hours the growth on the plate of 10^{-4} dilution was dense enough that there were no separate colonies to identify. On the plate of 10^{-5} dilutions was to identify the colonies of microbes and growth on 10^{-6} plates was not enough visible. Two colonies were marks as" S1 & S2" to use the pure culture preparation; these colonies are shown in Figure 1 & 2.

Figure 1: Colony of Sample A (S1)

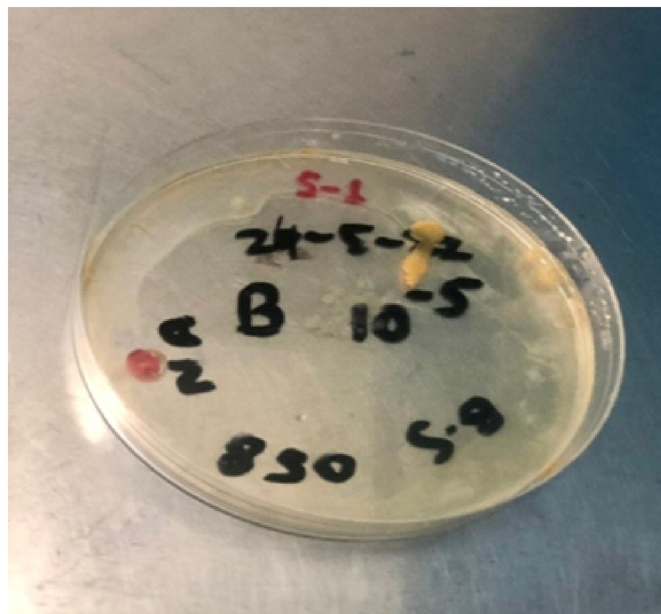

Figure 2: Colony of Sample (S2)

Marked colonies of bacteria were used to form pure culture. The inoculating loop is red hot to make is sterile then allowed it to cool. After cooling the loop is slightly touched to the marked colony and the streak on the media plate. Same method was repeated for next colony. The plates were incubated at 37°C for next 48 hours and the microbes were allowed to grow. After this incubation the pure culture of the bacteria was available for the further processing. Pure culture of colonies S1 and S2 are shown in Figure 3.

Figure 3: Pure Culture of Colonies S2 & S1

Broth media was prepared by adding the weight components of it in distilled water. The components dissolved by shacking. After the preparation of broth media, it was autoclaved to kill the microbes that could contaminate it. Components of broth media that was prepared are shown in Table 4.

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Table 4: Ingredients of Broth Media

Ingredients	Per 1L (g)	Per 500mL (g)
Yeast Extract	3.0	1.5
Peptone	5.0	2.5
Sodium Chloride	8.0	4.0

10 ml of broth added to the falcon tubes, sterile inoculating loop is used to pick the growth from plate and added to the broth. Then the broth culture was incubated at 37°C. A control broth falcon tube was also used to detect growth of microbes. After the incubation the broth culture became dense that was sign of microbial growth in it. In this way pure inoculums of S1 and S2 were available to check the antibiotic efficacy of drugs, that is shown in Figure 4.

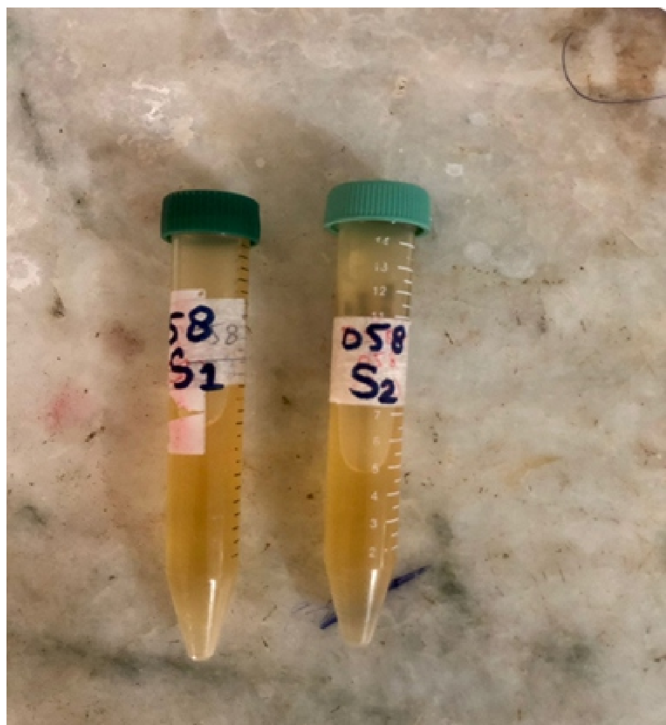

Figure 4: Pure Inoculums of Colonies S1 & S2

A specific quantity of each drug was used to form the solution. The weighed amount of each antibiotic drug was added to the known quantity of the distilled water in test-tubes. The test-tubes were shacked to dissolve the un-dissolved components of drugs and to make the solutions homogenized. Solution of each drug was made by the ratio of 5mg/1ml. As every drug has a specific amount of active compound that is responsible for its activity and mentioned on the package and drug has a higher total weight. 50mg of active compound were used to make the solution in 10ml of distilled water. So, 50 multiplied by the ratio of total weight of drug to active compound to get the quantity of drug containing 50mg of active compound. Total weight of drugs weight of active compound in drugs and weight use to form solutions are mentioned in Table 5.

Table 5: Weight of Drugs (Total, Active Compound, Ratio, Used)

Drug #	Total Weight of Drug	Weight of Active Compound	Ratio T.D: A.C	Weight of Drug Used
D1(Moxifloxacin)	692mg	400mg	1.73: 1mg	86.5mg
D2(Cephalexin)	520mg	500mg	1.04: 1mg	52mg
D3(Levofloxacin)	823mg	500mg	1.64: 1mg	82.3mg
D4(Amoxicillin)	600mg	500mg	1.2: 1mg	60mg
D5(Cefixime)	512mg	400mg	1.28: 1mg	64mg

Calculated amounts of drugs having 50mg of active drug were weighted and added to the test-tubes. 10ml of distilled water was added to each tube and then shake to dissolve or mix-up the drug in water. In these ways' solutions of drugs 5mg/1ml were prepared that are shown in Figure 5.

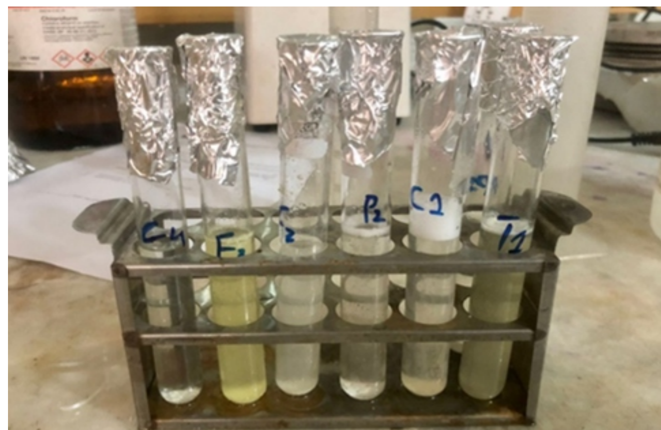

Figure 5: Solutions of Antibiotics

Small circular discs of paper of almost 5mm were prepared. These discs were autoclave to make them sterile. Each disc dipped in the antibiotic solution and placed at the center of media plate. Plates containing the discs dipped in antibiotics solution ready for inhibiting the growth of microbes. Media plates having discs containing antibiotics in their center were inoculated with the microbes. 100ul of S1 & S2 inoculum spread over the media and incubate at 37°C. After the incubation the growth was present but outside of the Zone of Inhibition of antibiotics.

RESULTS

All the antibiotics had different antibiotic activity against the strain S1 and S2 and the exact value (diameter) of Zone of Inhibition. The Zones of Inhibition were visible and the diameter of each zone measured with scale that provides information about the antibiotic efficacy of each drug. Zones of Inhibition that are formed of culture plates by different drugs used on S1 and S2 strains are shown in Figure 6 and 7.

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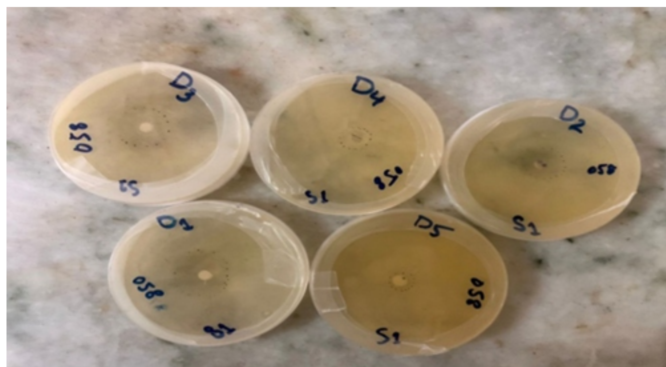
Figure 6: Zones of Inhibition against S1

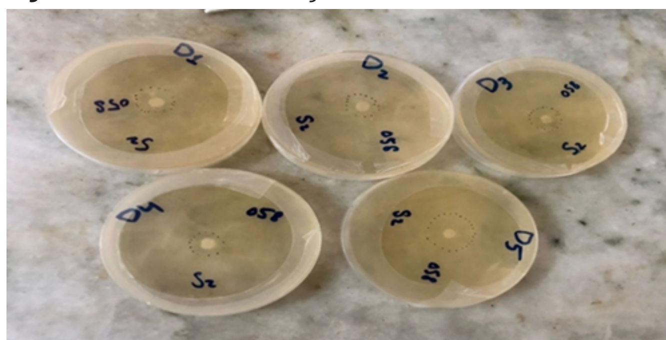
Figure 7: Zones of Inhibitions against S2

All the drugs are found efficient to variable extent against the indigenous microbes used in our experiment. Measurement of Zone of Inhibition against S1 & S2 are shown in Table 6 & 7.

Table 6: Zones of Inhibition against S1

Drugs	Diameter (mm)
Rifampicin (C)	27
D1	24
D2	19
D3	22
D4	12
D5	08

Table 7: Zones of Inhibition against S1

Drugs	Diameter (mm)
Rifmpacin (C)	27
D1	16
D2	17
D3	16
D4	13
D5	21

DISSCUSION

Previous studies have highlighted the significance of antibiotic-induced oxidation in bacteria, which plays a crucial role in the effectiveness of bactericidal medicines. This viewpoint aligns with our research findings pertaining to the effectiveness of antibiotics against autochthonous bacterial species. The findings of our experiment provide evidence in favor of the hypothesis that antibiotics possess the ability to impede the growth of these microorganisms. This inhibition may be attributed to a range of mechanisms, such as the disruption of cell wall integrity or the alteration of structural components. Recent study by Stokes *et al.*, developed on these observations by presenting more accurately that antibiotic-induced oxidation in bacteria contributes to bactericidal efficacy of drug [20]. The antibiotics drugs do not inhibit DNA replication machinery as a primary target also degrades the cell wall of microbes or denaturation of their structural component [21]. Our research, similar to earlier studies, provides evidence that antibiotics have varying effectiveness against particular types of bacteria. The observed zones of inhibition in our experiment exhibit variability among different antibiotics and bacterial strains, which is consistent with prior research findings. The aforementioned variability emphasizes the significance of customizing antibiotic therapy to the particular microbial species implicated, along with the notion of antibiotic specificity emphasized in prior studies.

CONCLUSIONS

In conclusion, indigenous bacteria can spoil fruits and potentially lead to various human diseases and infections upon consumption. Antibiotics serve as essential tools for combating bacterial infections by inhibiting or killing these microbes. Our research involved the isolation of these spoilage-causing microbes from fruit samples, effectiveness against these followed by testing the efficacy of commercially available antibiotics against them. Most antibiotics demonstrated isolated microbes, albeit to varying degrees. The success of antibiotic treatment depends on factors such as the purity of the drugs, their specificity to the target microbes, appropriate dosage, and treatment duration. Ensuring the responsible and judicious use of antibiotics can optimize their effectiveness in treating foodborne infections and preserving public health.

Authors Contribution

Conceptualization: MW
Methodology: FI, AI, RTM
Formal analysis: MW, A1
Writing-review and editing: AI, RTM, FI, SWU, RHU, MA,

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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REFERENCES

[1] Agriopoulou S, Stamatelopoulou E, Sachadyn-Król M, Varzakas T. Lactic acid bacteria as antibacterial agents to extend the shelf life of fresh and minimally processed fruits and vegetables: Quality and safety aspects. Microorganisms. 2020 Jun; 8(6): 952-75. doi: 0.3390/microorganisms8060952.

[2] Vallavan V, Krishnasamy G, Zin NM, Abdul Latif M. A review on antistaphylococcal secondary metabolites from basidiomycetes. Molecules. 2020 Dec; 25(24): 5848. doi: 10.3390/molecules25245848.

[3] Huang C, Feng S, Huo F, Liu H. Effects of four antibiotics on the diversity of the intestinal microbiota. Microbiology Spectrum. 2022 Apr; 10(2): 01904-21. doi: 10.1128/spectrum.01904-21.

[4] Altekruse SF and Swerdlow DL. The changing epidemiology of foodborne diseases. The American journal of the medical sciences. 1996 Jan; 311(1): 23-9. doi: 10.1016/S0002-9629(15)41627-1.

[5] Bhale UN. Survey of market storage diseases of some important fruits of Osmannabad District (MS) India. Science Research Reporter. 2011 Sep; 1(2): 88-91.

[6] Akinmusire OO. Fungal species associated with the spoilage of some edible fruits in Maiduguri Northern Eastern Nigeria. Advances in Environmental Biology. 2011 Jan; 5(1): 157-61.

[7] Rawat S. Food Spoilage: Microorganisms and their prevention. Asian journal of plant science and Research. 2015; 5(4): 47-56.

[8] Lobritz MA, Belenky P, Porter CB, Gutierrez A, Yang JH, Schwarz EG, *et al*. Antibiotic efficacy is linked to bacterial cellular respiration. Proceedings of the National Academy of Sciences. 2015 Jul; 112(27): 8173-80. doi: 10.1073/pnas.1509743112.

[9] Beuchat LR. Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. Microbes and Infection. 2002 Apr; 4(4): 413-23. doi: 10.1016/S1286-4579(02)01555-1.

[10] Kumar R, Pandey S, Kapoor P, Awasthi S, Bhatnagar T. Isolation and characterization of endemic strains of Lactobacillus sp. and evaluation of their probiotic activity. International Journal of Current Microbiology and Applied Sciences. 2014 Jan; 3: 907-16.

[11] Hedberg CW, MacDonald KL, Osterholm MT. Changing epidemiology of food-borne disease: a Minnesota perspective. Clinical Infectious Diseases. 1994 May: 671-80. doi: 10.1093/clinids/18.5.671.

[12] Lopatkin AJ, Stokes JM, Zheng EJ, Yang JH, Takahashi MK, You L, *et al*. Bacterial metabolic state more accurately predicts antibiotic lethality than growth rate. Nature Microbiology. 2019 Dec; 4(12): 2109-17. doi: 10.1038/s41564-019-0536-0.

[13] Nisa M, Dar RA, Fomda BA, Nazir R. Combating food spoilage and pathogenic microbes via bacteriocins: A natural and eco-friendly substitute to antibiotics. Food Control. 2023 Feb; 149: 109710. doi: 10.1016/j.foodcont.2023.109710.

[14] Levy SB and Marshall B. Antibacterial resistance worldwide: causes, challenges and responses. Nature medicine. 2004 Dec; 10(12): 122-9. doi: 10.1038/nm1145.

[15] Pimentel TC, de Oliveira LI, Macedo ED, Costa GN, Dias DR, Schwan RF, *et al*., Understanding the potential of fruits, flowers, and ethnic beverages as valuable sources of techno-functional and probiotics strains: Current scenario and main challenges. Trends in Food Science & Technology. 2021 Aug; 114: 25-59. doi: 10.1016/j.tifs.2021.05.024.

[16] Rani A, Saini KC, Bast F, Varjani S, Mehariya S, Bhatia SK, *et al*., A review on microbial products and their perspective application as antimicrobial agents. Biomolecules. 2021 Dec; 11(12): 1860-81. doi: 10.3390/biom11121860.

[17] Cook MA and Wright GD. The past, present, and future of antibiotics. Science Translational Medicine. 14, eabo7793. doi: 10.1126/scitranslmed.abo7793.

[18] Reyes-Estebanez M, Sanmartin P, Camacho-Chab JC, Susana C, Chan-Bacab MJ, *et al*., Characterization of a native Bacillus velezensis-like strain for the potential biocontrol of tropical fruit pathogens. Biological Control. 2020 Feb; 141: 104127. doi: 10.1016/j.biocontrol.2019.104127.

[19] Muhammad S. Shireen. Antibacterial Approach of Lactobacillus Species Isolated from Exterior of Fruits. Journal of Biomedical and Allied Research. 2021; 2(2): 1-9. doi.org/10.37191/Mapsci-2582-4937-2(2)-022.

[20] Stokes JM, Lopatkin AJ, Lobritz MA, Collins JJ. Bacterial metabolism and antibiotic efficacy. Cell Metabolism. 2019 Aug; 30(2): 251-9. doi.org/10.1016/j.cmet.2019.06.009.

[21] Fan XY, Tang BK, Xu YY, Han AX, Shi KX, Wu YK, *et al*., Oxidation of dCTP contributes to antibiotic lethality in stationary-phase mycobacteria. Proceedings of the National Academy of Sciences. 2018 Feb; 115(9): 2210-5. doi: 10.1073/pnas.1719627115.

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