SURVIVAL ASSESSMENT OF PATHOGENIC BACTERIA WITH ANTIBIOTIC RESISTANCE
TRAITS FROM FRESH SUMMER ROYAL GRAPES: IN VITRO MICROBIAL CHALLENGE TEST


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ABSTRACT

Table grape act as a popular and mouthwatering fruit supplement over the world. Unhygienic handling of these fruits can possess a diverse threat to public health safety. Moreover, the fruit juice can serve as a nutrient for bacterial growth as well if kept in an unsanitary condition. For this purpose, five imported grape samples (Red Globe, Perlette, Calmeria, Princess and Summer Roya) were tested to assess the presence of pathogenic bacteria and drug-resistant pattern of the isolates. The total bacterial count (TBC) and total fungal count (TFC) showed the presence large amount of bacterial (ranged from 4.4 x 10^7 to 8.1 x 10^7) and fungal population on the grape samples, especially on the skin part of the fruits. Several pathogenic isolates, Escherichia coli, Staphylococcus spp., and Pseudomonas spp. were isolated from tested grape samples. However, the presence of Salmonella spp., Shigella spp., Vibrio spp. and Klebsiella spp. were not found from any of the samples. The concerning part of the present study was drug-resistant bacterial strains isolated from supplied Fresh Summer Royal Grape. The most of the isolated bacterial spp. were resistant to all most all of the commonly used antibiotics such as ampicillin (10 µg), trimethoprim/sulfamethoxazole (25 µg), tetracycline (30 µg), ceftriaxone (30 µg), imipenem (100 µg), chloramphenicol (30 µg), azithromycin (15 µg), novobiocin (30 µg), vancomycin (30 µg), penicillin G (10 µg), ciprofloxacin (5 µg), neomycin (30 µg), levofloxacin (5 µg), kanamycin (30 µg) and cefixime (5 µg). No grape samples showed any kind of antimicrobial activity against both pathogenic and non-pathogenic bacteria. The survival pattern of inoculated bacterial samples in the grape sample showed a slight decrease in growth in the course of time.

Keywords: Summer Royal grape, Grape-borne bacteria, Pathogenic Microorganisms, Antibiotic susceptibility, Microbial challenge test.

INTRODUCTION

Grape, Vitis vinifera, are the most common fruits all over the world since centuries ago. They are believed to be originated from Northwest Asia and have been cultivated in many regions of Central Asia, Australia, the United States of America and countries of Europe (Samad et al., 2012). Recently some tropical countries like Thailand and Malaysia have started to cultivate grape, while Bangladesh is now under trial to accomplish the success of grape cultivation. Grapes contain susceptible antioxidant that minimize multiple disease risks (Fatema et al., 2013). The fruits like grapes belong to the berry family; constitute a cluster or bunch of grapes. It has many nutritional, healthy and medicinal properties and broadly accepted as one of the most edible foods to the trenchermen. Grape contains 82% water, 12–18% carbohydrates, 0.5–0.6% proteins and 0.3–0.4% fat. Therefore, it’s a good source of potassium, vitamin A, vitamin C, calcium and phosphorus. It contains the significant amount of all those elements; 0.1–0.2%; 0.001–0.0015%; 0.01–0.02%; 0.01–0.02% and 0.08–0.01% respectively (Mukesh Y. et al., 2009). Nutrients like boron are also found in grapes (Demir & Serindag et al., 2006), which are a possible element for bone health and bone marrow establishment (Mukesh Y. et al., 2009). One of the major nutritional value including 78 calories of energy, protein (0.3 g), carbohydrate (19 g), 3% calories from fat (0.23 g), sodium (0.18 mg), potassium (155 mg), fibre (0.4 g), calcium (20 mg), phosphorus (30 mg) and vitamin C (1 g) have been yielded from per halves of grape. Furthermore, phytochemicals like gallic acid, catechin, and epicatechin are originated from skin and seeds of grape and these phytochemicals have been reported as the raw substrates to produce antioxidative dietary supplements (Yilmaz & Toledo et al., 2004; Mukesh Y. et al., 2009). White grape pomace could yielded antioxidant thioconjugates (Selga et al., 2004, Mukesh Y. et al., 2009). Many researchers proved that Concord grape juice plenty amount of flavonoids, which has the significant antioxidant potency with astrocortin (vitamin E) (O’Byrne et al., 2002, Mukesh Y. et al., 2009). Flavonoid effectively works as a strong antioxidant and have the ability to protect the host from oxidative stress. Also, free radical damages and onslaught of chronic diseases have been decreased by Concord grape juice. Surprisingly, dried grape seeds have flavan content with high antioxidant activity; acquired after the colour extraction and alcohol distillation of wine production even after exposure to advanced temperatures (Gonzalez - Paramas & Rivas – Gonzalo et al., 2004, Mukesh Y. et al., 2009). Although, grapes containing, all the isolating compounds are not medically significant, very few of them having biological or medicinal activities. Anticarcinogenic, antioxidant, antidiabetic, anti-atherogenic, neuroprotective, anti-ageing, anti-obesity, immunomodulatory and anti-infection are the constituents of medicinal properties of grape (Mukesh Y. et al., 2009) (Figure 1). Particularly, more evident biological activities of resveratrol have been extracted from the skin and seeds of the grape.
Two individual grapes include Table grape and Wine grape, are consumed; Table grapes are eaten up fresh, utilized to prepare jam and used in recipes. Grape is considered as a pack of nutrients, such as sugars, mineral salts, enzymes, vitamins and phytochemicals which give the sensory properties of wine (Waterhouse et al., 2002 and Walzem et al., 2008). Grape products are rich in phenolic compounds which draw an attraction because of their beneficial health benefits (Fuleki & Ricardo-da-Silva, 2003 and O’Byrne et al., 2002). Several antioxidants, namely flavonoids, epicatechin, anthocyanin, carotenoids are present in high amount in grape extract (Lopez-Velez et al., 2003; Park et al., 2003 and Viljanen et al., 2002). Multiple clinical examinations reported the benefit of consumption of fruit with high phenolic content, especially resveratrol, by decreasing the risk of developing cardiovascular disease (Garcia-Alonzo et al., 2004 and Bertelli & Das, 2009). Furthermore, grape seed extract has been promulgated to lower the risk of several cancer and Alzheimer’s disease as well (Marambaud et al., 2005).

Grapes are considering as a full of useful and essential chemicals, however it’s seed and skin are habitually contains the growth and survival properties of diverse types of pathogenic and non-pathogenic bacterial populations including B. subtilis, E. coli, Pseudomonas aeruginosa, Bacillus cereus, Staphylococcus aureus, Listeria monocytogenes (Jayapraakash et al., 2003), Rhodes et al., 2006 and Fernandez et al., 2007). Though the antimicrobial activity of the grape extract against E. coli O157:H7 have been reported already (Kim et al., 2008), but several contaminating factors can spoilage the grapes during the cultivation and post-harvesting process by contacting with soil, air, water or animal wastes (Chen et al., 2004).

Inadequate sanitizing practices in the shop and retail markets are one of the leading sources of microbial contamination (Olaimat & Holley et al., 2012). Presence of pathogenic bacteria such as Escherichia coli, Salmonella spp., and Stigella spp. has possessed a possible threat to the consumer creating foodborne illness. (Eul et al., 2010 and Aysicek et al., 2006). In addition, drug-resistant organisms are increasing swiftly due to consumption of unhygienic raw fruits, triggering health-related concern and subsequently hampering the disease medication (Dutta & Noor et al., 2013 and Noor & Acharjee et al., 2013). However, implementing a new method would be highly preferable to check overall food quality and safety issues. The microbial challenge test is one of them and can assess microbial potential of certain foods through inoculating specific microbial communities under the diverse regulated environmental conditions. The main focusing point of such kind of in vitro microbial challenge test is to identify growth potential and to evaluate the highest growth rate of particular microbes. (Fatema et al., 2013). Thus, consumer safety must be considered by testing of food stability as well. Till now several accessions have been done to evaluate the surviving capability of microorganisms associated with grape samples, though, which are still insufficient. Moreover, previous researches were mostly focused on the grape extract or grape seed extract. As peoples of our country commonly take grape as whole fruit and not take the extract as food supplanter, the quality of the whole fruit assessment is required for public health safety. Based on these disclosed facts, the present study is designed (1) to isolate and identify drug-resistant microbes that associated with grape spoilage activities, (2) to unfold antimicrobial activity of the tested grape samples, and eventually (3) to investigate the survival capabilities of the grapes spoiling microflora by performing microbial challenge test (an efficient in vitro testing to check the viable count of microbes).

MATERIAL AND METHODS

Study area, sampling and sample processing

The present study was carried out with five groups of imported grape (Vitis vinifera) samples with Red Globe, Perlette grape, Calmeria grape, Princess and Summer Royal grape, inventing from the following locations Japan, Peru, New Zealand, Sparta and West Virginia, consecutively. Random samples were taken haphazardly from different supermarkets sites in Dhaka, Bangladesh, within a period from November 2019 to December 2019 maintaining usual protocol (APHA, 1998). For the isolation and identification of grape spoiling microbiome and mycobioime, a standard procedure was maintained taking 10g of grape sample mixed with 90 ml peptone water, the pH was adjusted 7.2 ± 0.2 and diluted up to 10^5 fold.

Isolation and enumeration of spoiling microorganisms

To test the presence of entire viable microorganisms, presence in grape samples, conventional spread plate technique was performed by taking 0.1 mL of each diluted sample spread onto Nutrient Agar (NA) and Sabouraud Dextrose Agar (SDA) plates (Cappuccine & Sherman et al., 1996). Therefore, these plates were observed after 24 hours incubation at 37 °C and 48 hours incubation at 25 °C for total viable bacteria bacterial count and fungal count, respectively. 0.1 mL sample was collected from 10-3 and 10-5 diluted samples and extent onto MacConkey agar for the enumeration coliforms (E. coli and Klebsiella spp.) and MFC media for fecal coliforms, respectively. Later, the agar plates were incubated for 24 hours at 37°C but for the faecal coliforms, the approximate temperature was 44.5 °C with carried out the random period e.g. 24 hours. To characterized the E. coli strains, green metallic sheen colony were observed on Eosin methylene blue (EMB) agar medium. For isolation of Staphylococcus spp. and Pseudomonas spp. Mannitol Salt Agar (MSA) and Pseudomonas agar were used respectively, and 0.1 mL of diluted sample was spread onto these agar plates and incubated for 24 hours at 37 °C. Also, 0.1 ml of suspension was extent onto the Listeria identification media and plates for enumeration of Listeria spp. and incubated for 24 hours at 37 °C. Listeria spp. identification
was listing out by observing blue-green colonies on Listeria identification agar media. However, all those bacterial isolates were biochemically tested for further confirmation (Table 2) (Fatema et al., 2013).

Enrichment of bacterial strains for enumeration

Enrichment culture is important for viable but non-culture able (VBNC) bacterial cells or stressed cells to increase their number in detectable level for proper enumeration. (Rahman & Noor, 2012 and Fatema et al., 2013). For this reason, in the present experiment to enrich Salmonella, Shigella and Vibrio spp., 1 mL of each broth sample was added into 9 mL of Alkaline Peptide Water (APW) and Selenite Cystine Broth and incubated for 6 hours at 37 °C. After incubation, to isolate Vibrio, Salmonella, Shigella spp., two common isolating agar plates such as Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar media and Salmonella Shigelisa (SS) agar media for those bacterial species were used respectively. After that, both plates were kept into the incubator at 37 °C for 48 hours to get the detectable colonies. Eventually, all the finding isolates were observed biochemically by maintaining standard procedure (APHA, 1998 and Fatema et al., 2013).

Antibacterial susceptibility test

All the bacterial isolates were spread on Muller–Hinton agar media to test the susceptibility against 15 commonly antibacterial drugs (including first, second and third-generation drugs) by using disc diffusion method (Difco, Detroit, MI) and following the typical protocol (Fatema et al., 2013). Antibiotic discs contained ampicillin (10 µg), trimethoprim/sulfamethoxazole (25 µg), tetracycline (30 µg), ceftriaxone (30 µg), imipenem (10 µg), chloramphenicol (30 µg), azithromycin (15 µg), novobiocin (30 µg), vancomycin (30 µg); penicillin G (10 µg), ciprofloxacin (5 µg), neomycin (30 µg), levofloxacin (5 µg), kanamycin (30 µg) and cefixime (5 µg).

Determination of antibacterial activity of grape samples

The Agar Well Diffusion Method is a proven scientific method to test the antibacterial activity of grape sample which was previously used by (Hussain et al., 2010 and Fatema et al., 2013). All the tested organisms (Bacillus spp., Pseudomonas spp., Vibrio spp., Listeria spp., Salmonella spp., Klebsiella spp., E. coli and Staphylococcus aureus) were put into the normal saline to make a suspension comprising of 10^6 cells/mL (compared with McFarland standard) after that the lawns were set on Muller–Hinton agar (MHA) plates separately. After drying, sterile cork borers were used to form 8 mm wells. A volume of 100 µL of each sample blend (with a concentration of 11 µg/μL) was transferred into separate wells, dried and subsequently incubated at 37 °C for 12-18 hours. Normal saline and Gentamicin (10 µg) were used as a positive and negative control, respectively. The occurrence of a clear zone around the sample solution (if any) denoted the existence of antibacterial activity (Adib et al., 2008 and Fatema et al., 2013).

Microbial challenge test

Samples processing

The surface of the one chosen grape samples (Summer Royal) were washed for five times by 90% ethanol to free from the microbial association. Afterwards, 10 gm samples were mixed with 90 mL buffered peptone water (BPW), and then from the resultant suspensions 10 mL each sample aliquots with buffered peptone water, eventually centrifuged at 5000 rpm for 5 minutes for three times, using with 90% alcohol centrifuged for two times and finally centrifuged was performed for 70% alcohol (Fatema et al., 2013). The resultant pellets after centrifugation steps were collected and suspended with 9 mL BPW. From the suspended solution 100 μL of each sample was poured on to the nutrient agar media to test the remaining growth for getting contamination-free microbial growth. The culture plates were kept in the normal refrigerator at -4 °C until further use (Fatema et al., 2013).

Microbial inoculation and growth count

One loop full pure culture of each sample (Bacillus spp., Pseudomonas spp., Vibrio spp., Listeria spp., Salmonella spp., Klebsiella spp., E. coli and Staphylococcus aureus) were poured into 9 mL sterile normal saline containing test tubes (Cappuccino & Sherman, 1996). After 6 hours of incubation at 37 °C (Fatema et al., 2013), the cell density was moved to around 10^5 cfu/g (compared with McFarland Standard OD 0.5) for each test suspension. Microbial challenge test was performed for every bacterial isolate found in grape samples. 10 mL of suspension from each grapes sample was inoculated with 100 µL of the bacterial suspension made the initial load of ~10^5 cfu/g, and the mixture was incubated at 37 °C for 15 days. No inoculation was done for control samples. Finally to examine the growth pattern of the isolates, the prepared suspensions were diluted up to 10^6 folds and spread onto Luria Bertani (LB) agar media, which then observed after every 24-hour interval according to standard spread plate technique (Cappuccino & Sherman, 1996 and Fatema et al., 2013).

Assessment of reduction in bacterial load

For the finding of the probable growth rate of artificially inoculated bacterial species in the samples considered, the log reduction and percent reduction were analyzed using the following formulas (AFSSA, 2008 and Fatema et al., 2013). Log10 reduction = Log10 (initial load) – Log10 (final load) % Reduction = [(Log10 (initial load) – Log10 (final load)/Log10 (initial load))] x 100. The standard initial load at 0 hour of inoculation is generally considered 10^6 cfu/g, though in the present study the maximal load after 24–48 hours was also taken into account instead of the initial load stated above. Deducing the final load from the maximum growth point would conclude the accurate decay pattern of microorganisms (Fatema et al., 2013).

Statistical analysis

All the experimentations were performed individually 3 times. Every values were analysed statistically to determine the p-value done by t test (Shahriar et al., 2019).

RESULTS

Microbial prevalence in the tested grape samples

The present experiment is to demonstrate consistent results since a vast amount of microorganisms including bacteria and fungi have been isolated from the tested grape samples (Table 1). Pseudomonas spp. was found to be the predominant bacterial growth on each tested samples. Staphylococcus spp. was prevalent on four samples except for the Perlette grape. Escherichia coli growth was found to be variable
Staphylococcus spp. were subject to several biochemical tests for further identification. Results are demonstrated in Table 2. After incubation no growth of fecal coliform, Staphylococcus, Shigella, Salmonella and Vibrio spp. were absent on any of the samples. Surprisingly, in the skin area of the tested samples, the number of microbial growth was higher compare to flesh portions. (Table 1).

Drug resistance pattern of the bacterial isolates

Study of antibiogram revealed that few species show sensitivity towards imipenem (10 µg), cefiximycin (5 µg), and kanamycin (30 µg) (Figure 2). Staphylococcus species were found to be resistant against all the antibiotics except sulphamethoxazole-trimethoprim (25 µg) (Figure 2). A similar scenario was found in the case of Pseudomonas with the exception of sensitivity to imipenem (10 µg) and ceftriaxone (30 µg) (Figure 2). All most every isolate was shown a multi-drug resistance pattern (Figure 2).

Antibacterial activity of grape samples

Our experiment did not reveal any bactericidal activity of any of the grape sample tested. However, the organic solvent extracts of grape juice show this antibacterial action, where we used only the crude fraction of the grape samples.

In vitro challenge test to observe bacterial survival capability

After 48 hours incubation of Summer Royal Grape samples with each of the 6 tested bacteria, an initial escalation pattern of microbial growth was observed, especially in terms of E. coli where, the maximal microbial growth points were calculated after 15 days later compare with final microbial load in Summer Royal Grape samples (Figure 3, A-F), the growth of Pseudomonas spp., were reduced more than 2 log whereas the reduction score for E. coli, Salmonella spp., Bacillus spp., and Klebsiella spp., were nearly 2 log. Even for the least isolate (Staphylococcus spp.), the reduction score was observed approximately 1-log (Figure 3).

Assessment of reduction in bacterial load

Interestingly, the reduction score for Staphylococcus spp. were estimated more than 3-log in the Summer Royal Grape samples (Figure 4). Constant percentage of reduction trend was observed for Staphylococcus spp. which was higher in the Summer Royal Grape samples (Figure 4). In Summer Royal Grape samples, the growth of Salmonella spp., Klebsiella spp., and E. coli were reduced more than 2-log whereas, Bacillus spp., and Pseudomonas spp., were reduced above 1-log (Figure 4). From Figure 4, a slight change in bioburden was observed, especially in terms of Salmonella spp., and E. coli. The minor reduction was also determined for Staphylococcus spp., Bacillus spp., Klebsiella spp., and Pseudomonas spp., nevertheless, the reductions were very insignificant and hence the overall findings are evidently suggestive of grape to be supportive for microbial growth.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Part</th>
<th>TVC (cfu/g)</th>
<th>Fungi (cfu/g)</th>
<th>E. coli (cfu/g)</th>
<th>Staphylococcus (cfu/g)</th>
<th>Pseudomonas (cfu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red Globe</td>
<td>Skin</td>
<td>7.6 x 10⁶</td>
<td>5.0 x 10⁴</td>
<td>0</td>
<td>2.7 x 10⁴</td>
<td>3.2 x 10⁷</td>
</tr>
<tr>
<td></td>
<td>Flesh</td>
<td>1.2 x 10⁶</td>
<td>8.0 x 10⁵</td>
<td>0</td>
<td>3.0 x 10⁵</td>
<td>1.0 x 10⁸</td>
</tr>
<tr>
<td>Perlette</td>
<td>Skin</td>
<td>2.0 x 10⁶</td>
<td>1.0 x 10⁴</td>
<td>8.2 x 10⁵</td>
<td>0</td>
<td>2.6 x 10⁷</td>
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<tr>
<td></td>
<td>Flesh</td>
<td>1.8 x 10⁵</td>
<td>7.0 x 10³</td>
<td>3.0 x 10²</td>
<td>0</td>
<td>4.9 x 10⁹</td>
</tr>
<tr>
<td>Calmeria</td>
<td>Skin</td>
<td>5.1 x 10⁵</td>
<td>2.6 x 10⁴</td>
<td>0</td>
<td>7.1 x 10⁴</td>
<td>3.2 x 10⁷</td>
</tr>
<tr>
<td></td>
<td>Flesh</td>
<td>4.8 x 10⁵</td>
<td>2.4 x 10⁴</td>
<td>0</td>
<td>1.0 x 10²</td>
<td>4.8 x 10⁷</td>
</tr>
<tr>
<td>Princess</td>
<td>Skin</td>
<td>8.3 x 10⁵</td>
<td>1.7 x 10³</td>
<td>9.0 x 10²</td>
<td>4.2 x 10³</td>
<td>1.1 x 10⁴</td>
</tr>
<tr>
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<td>8.8 x 10⁵</td>
<td>1.8 x 10⁴</td>
<td>0</td>
<td>3.6 x 10⁴</td>
<td>1.5 x 10⁷</td>
</tr>
<tr>
<td>Summer Royal</td>
<td>Skin</td>
<td>8.1 x 10⁵</td>
<td>1.6 x 10³</td>
<td>0</td>
<td>5.1 x 10⁶</td>
<td>3.28 x 10⁷</td>
</tr>
<tr>
<td></td>
<td>Flesh</td>
<td>4.4 x 10⁵</td>
<td>1.0 x 10³</td>
<td>0</td>
<td>1.5 x 10⁷</td>
<td>4.3 x 10⁷</td>
</tr>
</tbody>
</table>

** After incubation no growth of fecal coliform, Salmonella, Shigella, Klebsiella and Vibrio spp. was monitored. All the isolates on selective medium were subject to several biochemical tests for further identification. Results are demonstrated in Table 2.
### Table 2: Biochemical test results of the isolated bacterial species

<table>
<thead>
<tr>
<th>Sample</th>
<th>Triple Sugar Iron Agar</th>
<th>MR</th>
<th>VP</th>
<th>Indole</th>
<th>Citrate</th>
<th>Mobility</th>
<th>Catalase</th>
<th>Oxidase</th>
<th>Suspected organism</th>
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</thead>
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<tr>
<td></td>
<td>Slant</td>
<td>Butt</td>
<td>Gas</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red Globe</td>
<td>A</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>Staphylococcus spp.</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>Pseudomonas spp.</td>
</tr>
<tr>
<td>Perlette</td>
<td>A</td>
<td>K</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>Staphylococcus spp.</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>K</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>Pseudomonas spp.</td>
</tr>
<tr>
<td>Calmeria</td>
<td>A</td>
<td>K</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Pseudomonas spp.</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>A</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>Princess</td>
<td>A</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Staphylococcus spp.</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>K</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>Pseudomonas spp.</td>
</tr>
<tr>
<td>Summer Royal</td>
<td>A</td>
<td>K</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Pseudomonas spp.</td>
</tr>
</tbody>
</table>

**Figure 2**: Resistant and susceptible pattern of (A) *E.coli*, (B) *Pseudomonas spp.*, (C) *Staphylococcus spp.* against different types of antibiotics. All experiments were carried out three times with 95% accuracy and presented data ware statistically analyzed by showing the standard errors consider as 5%.
Figure 3 Bacterial Survival of in Summer Royal Grape sample (A - F). The turbidity of bacterial culture (A: *Escherichia coli*, B: *Klebsiella* spp., C: *Staphylococcus* spp., D: *Pseudomonas* spp., E: *Salmonella* spp., F: *Bacillus* spp.) were adjusted with the standard solution of McFarland (OD<sub>600</sub> 0.5) and then inoculate into the processed grape samples. Enumeration of bacterial growth was determined by the colony forming units of per gram of samples (cfu/g) on LB plates at every 24 hours interval up to 15 days.

Figure 4 Growth decay and survival capacity of the test isolate in Summer Royal Grape. Reduction in bacterial growth (CFU/gm) in Summer Royal Grape samples. A. Log10 reduction. B. % reduction.
CONCLUSIONS

Overall, the current experiment attempted to reduce the implication of foodborne illnesses of the consumers. Results from the present study distinctly contribute a complete bacteriological profile of imported grape samples, which has public health importance. Such an approach can be simulated by using resource-poor settings lacking extensive molecular biological studies on microbial virulence genes, microbial toxins or metabolites, and eventually the growth-promoting factors. Further analysis with the advanced experiment in different fruit items with the antibacterial trait of the alcoholic extract of grape juice would reveal the growth and social relationship of microbial communities compare to fruit types, which may avail to intuition the product shelf-life and the consumer safety.

Author contributions: This work was carried out in collaboration between all authors. All the authors have accepted responsibility for the entire content of this submitted manuscript and approved the submission. Authors TA, AS, MA, and TR performed experiments. TA, AS, MA, TR, and TBE conceived the study and designed the experimental procedures. TA, AS, MRM and TBE designed and planned the studies, supervised the experiments. TBE also acted for all correspondences. TA, AS, MRM and TBE participated in the manuscript draft and has thoroughly checked and revised the manuscript for necessary changes in format, grammar and English standard. All authors read and approved the final version of the manuscript. All authors read and approved the manuscript.

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Competing interest: The authors declare that they have no competing interest.

REFERENCES

Vitis vinifera


**Table Legends:**

Table 1: Bacterial and fungal growth on grape samples.
Table 2: Biochemical test results of the isolated bacterial species.

**Figure Legends:**

**Figure 1:** Medicinal properties and effective applications of grapes.
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