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Microbiological profile of potato samples collected from Bangladesh Agricultural Research Institute (BARI) and notification of anti-bacterial traits

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Present study assessed the microbiological quality of different categories of consumable potatoes collected from Bangladesh Agricultural Research Institute (BARI), the largest multi-crop research institute of the country conducting research on a large number of crops. Five samples each from 15 categories of potato samples (Lara, Quincy, Cardinal, Esprit, Meridian, Lady Rossetta, Astrix, Soikat, Red potato, White potato, Russet potato, Yellow flesh potato, Sweet potato, Purple potato and Petiets potato) were subjected to microbiological analysis through conventional culture followed by the biochemical identification tests of the pathogens. A huge array of bacterial and fungal contamination was noticed within a range of (~10^8 cfu/g) in almost all samples studied. Among the bacterial pathogens, *Escherichia coli* and *Staphylococcus* spp. were found to be predominant. Study of antibiogram revealed that most of the isolates were resistant against the commonly used one or multiple antibiotics. Finally, all the samples were also examined for presence of any anti-microbial activity against different pathogenic bacterial species. Astrix potato samples exhibited antimicrobial activity against *Shigella* spp and the Russet potato samples exhibited the anti-bacterial activity against *Staphylococcus* spp.

Key words: Potato; Microbiological quality; Drug-resistance; Antimicrobial activity; Consumer safety

Potato is one of the major and popular food items around the world (*Solanum tuberosum*) because of its high nutrient content as well as for its easy cultivation procedures (1-3). Production of potatoes at larger scale is necessary to convene the nutritional demands of huge population in developing countries like Bangladesh (2, 4). More than 3000 species of potato have been found in a large plant family of Solanaceae. On the basis of their production rate (325 thousand tons in 2007), potato is the third most important food crop after wheat and rice (5).

Because of the nutritional benefit, vegetables are being increasingly consumed and included in our daily meals almost every day (6-9). Potatoes, one of the most productive crops/ vegetables, have long been playing a significant role in ensuring food security globally. Bangladesh, an agro-based country, is well known to be the major potato producer in the South-East Asia. However, like other raw fresh vegetables, potatoes may also come in contact with an array of microorganisms including *Aeromonas* spp., *Bacillus cereus*, *Campylobacter jejuni*, *Clostridium botulinum*, *E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., *Shigella* spp., *Staphylococcus* spp. and *Vibrio cholerae*, resulting in various diseases (10-16). Therefore, survival and growth of pathogens in raw fresh vegetables are of paramount importance with respect to spread and transmission of diseases in humans and animals.

Potatoes are processed into many products including frozen, dried, ready-to-eat and minimally processed products. The potential risk of consumption of contaminated potato is lower than from other fresh vegetables because this commodity is cooked before eating. However, contaminated potato represents a potential risk of cross-contamination of other fresh vegetables in the processing plant and at home. Additionally, increased consumer demand for new and existing potato products highlights the importance of ensuring their microbiological safety (2, 4). Moreover, the concern for pathogens in vegetables including potatoes has risen due to the emerging outbreaks of food borne illness causative of the consumption of the minimally processed vegetables (17). Another health related issue lies over the antibiotic abuse which has been reported to extend the risk of acquiring resistance in the pathogens which badly affects the medication (18-22). The probability of dissemination of drug resistant pathogens in vegetables through contaminated water and/or other means is of great public health importance.

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Along these lines, present research attempted to detect and quantify total viable bacteria, total and fecal coliforms, and specific pathogens in the collected potato samples; to demonstrate the drug-resistance traits of the pathogenic isolates; and finally to investigate the antimicrobial activity (if any) of the potato sample of current interest.

MATERIALS AND METHODS

Study area, sampling and sample processing. Samples of 15 categories of healthy potato including Lara, Quincy, Cardinal, Esprit, Meridian, Lady Rossetta, Astrix, Soikat, Red potato, White potato, Russet potato, Yellow flesh potato, Sweet potato, Purple potato and Petiets potato were collected from Bangladesh Agricultural Research Institute (BARI) following standard protocol (23). Samples were quickly transported to the laboratory, and prior to microbiological assay, 10 g of each samples with 90 ml buffer peptone water (pH 7.2 ± 0.2) and the homogenized clear liquid was transferred into sterile airtight bottles (16). The homogenized samples were diluted up to 10^{-6} by following the standard methods of 10 fold dilution (24).

Enumeration of total viable bacteria and fungi. An aliquot of 0.1 ml from the dilution 10^{-3} and 10^{-6} of each sample was spread onto nutrient agar (NA) plate (Hi-Media Laboratories Pvt. Ltd., India) for enumerating total viable bacteria (TVB) and on Sabouraud Dextrose agar (SDA) plate (Hi-Media Laboratories Pvt. Ltd., India) for the estimation of fungal load, by means of spread plate technique (24). Plates were incubated at 37 °C and 25 °C for 24-48 hours for bacterial and fungal enumerations, respectively.

Estimation of total fecal coliform, *Escherichia coli* and *Klebsiella* spp. From the dilutions 10^{-3} and 10^{-6}, 0.1 ml of each sample was spread onto the membrane fecal coliform (MFC) agar and MacConkey agar (Hi-Media Laboratories Pvt. Ltd., India) for the enumeration of total fecal coliform (TFC), and coliforms (i.e., *Escherichia coli* and *Klebsiella* spp.), followed by incubation at 44.5 °C and 37 °C for fecal coliform and coliforms, respectively for 24 hours.

Estimation of *Salmonella* spp., *Shigella* spp., and *Vibrio* spp.. Enrichment was performed for Salmonella, Shigella and for Vibrio spp. in order to avoid the false negative results (16, 25, 26). After homogenization, 10 ml of samples were transferred into 90 ml of selenite cysteine broth (Oxoid Ltd., Basingstoke, Hampshire, England) and alkaline peptone water (APW) for the enrichment of *Salmonella*, *Shigella*, and *Vibrio* spp., respectively and incubated at 37 °C for 4-6 hours (16, 25, 26). Samples were then diluted up to 10^{-6} and 0.1ml of samples from each of the 10^{-3} and 10^{-6} dilutions were spread onto Salmonella-Shigella (SS) agar (Hi-Media Laboratories Pvt. Ltd., India) and thiosulfate citrate bile salt sucrose (TCBS) agar (Hi-Media Laboratories Pvt. Ltd., India) for the isolation of Salmonella spp. and Shigella spp., and Vibrio spp., respectively. Plates were incubated at 37 °C and the appearance of typical colonies (if any) was noticed within for 24-48 hours.

Isolation of *Staphylococcus* spp., *Pseudomonas* spp., and *Bacillus* spp. 0.1 ml from the dilution 10^{-3} and 10^{-6} was inoculated onto the mannitol salt agar (MSA) (Hi-Media Laboratories Pvt. Ltd., India), cetrimide agar plates (Hi-Media Laboratories Pvt. Ltd., India) and phenol red egg yolk polymyxin (MYP) agar base media (Oxoid Ltd., Basingstoke, Hampshire, England) following the spread plate technique and incubated at 37 °C for 24 hours for the isolation of *Staphylococcus* spp., *Pseudomonas* spp., and *Bacillus* spp., consecutively. Finally, the standard biochemical tests were performed to confirm the identification of all the pathogenic isolates found in all 15 types of categories of healthy potato samples by the previously described methods (24, 27, 28).

Antibiotic susceptibility test. The pathogenic isolates were examined for antibiotic susceptibility traits by disk diffusion assay on Mueller-Hinton agar (Difco Laboratories, Detroit, MI, USA) against commonly used antibiotics following the standard protocol (22, 28-31). Antibiotic disk (Oxoid Ltd., Basingstoke, Hampshire, England) used in the study included trimethoprime/sulfamethoxazole (25 µg/disc), erythromycin (15 µg/disc), amoxicillin (30 µg/disc), ceftriaxone (30 µg/disc), ciprofloxacin (5 µg/disc), streptomycin (10 µg/disc), ampicillin (10 µg/disc), tetracycline (30 µg/disc), chloramphenicol (30 µg/disc), cefixime (5 µg/disc), polymyxin B (300 units/disc), kanamycin (30 µg/disc), vancomycin (30 µg/disc), gentamicin (10 µg/disc), nalidixic acid (30 µg/disc), azythromycin (15 µg/disc) and penicillin G (10 µg/disc).

Determination of antibacterial activity of the potato samples. Agar well diffusion method was performed to determine the antibacterial activity of the potato samples (32, 33, 34). Individual bacterial pathogens (*Pseudomonas* spp., *Vibrio* spp., *Salmonella* spp., *Shigella* spp., *Klebsiella* spp., *Staphylococcus* spp., *E. coli*) were spread properly over the MHA agar plates using sterile cotton swab and wells were made in the MHA by cork borer. Each of the blended potato samples was then introduced separately in the specified well along with a positive control (antibiotic disk) and a negative control (normal saline). Presence of clear zone (if any) indicated the presence of antibacterial activity (34).

Statistical Analysis. All the experiments were performed in triplicate. Statistical analyses were performed by determining the P-value through t-test. Errors were also calculated (35).

RESULTS AND DISCUSSION

A large number of populations in developing countries like Bangladesh are always affected by some enteric diseases those are mainly caused by contaminated food and water (36). Therefore, for the consumers health safety, it is necessary to conduct studies which are directly and indirectly associated with microbiology of food and water. Bacterial proliferation including drug resistant ones in food items is responsible for serious health risk (37). Thus the present study may increment the vegetables research regarding our food quality.

Prevalence of microorganisms. All the potato samples studied were found to be highly contaminated with bacteria and fungi (Table 1). Specific microbial existence was further confirmed with biochemical identification tests (Table 2).

The presence of *E. coli* was observed within the range of 10^4 to 10^7 cfu/g in all samples expect Red, Russet and Petites samples. *Pseudomonas* spp. (10^5 to 10^7 cfu/g) was also detected in Lara, Cardinal, Esprit, Meridian, Lady Rossetta, Soikat samples. *Salmonella* spp. (10^6 to 10^7 cfu/g) was found in Lara, Quincy, Meridian and Petites samples, rest of the samples were free from the contamination with *Salmonella* spp. Cardinal, Esprit, Lady Rossetta, Red potato, White potato, Russet potato, Yellow flesh potato and Purple potato were found to be contaminated with *Shigella* spp. (10^5 to 10^7 cfu/g). *Vibrio* spp. was encountered (~10^5cfu/g) only in Quincy and Meridian. Among the 15 categories, only 2 categories of potato samples (Lara and Quincy) were contaminated with *Bacillus* spp. (10^5 to 10^6 cfu/g). All samples were found to be highly contaminated with *Staphylococcus* spp. (10^5 to 10^8 cfu/g). Only Astrix and Purples samples were found to be contaminated with *Klebsiella* spp. up to 10^7. Additionally, all the samples were found to be contaminated with fungal species within a range of 10^5 to 10^7cfu/g.

Cultivation of vegetables may largely account for such microbiological contamination. Manures used to promote the growth of vegetables may contain a large number of spoiling microorganisms (16, 38). Pathogens associated with untreated manure are assumed to enter into the food chain through crop. Thus, vegetables grown in such assistance of untreated fertilizers may play a significant role in showering pathogens to the consumers and therefore poses a great risk to public health (16). Moreover, the presence of microorganisms in potato samples studied might be largely due to the mishandling

TABLE 1. Bacterial load (cfu/g) in the tested potato samples

Sample	TVB	*E. coli*	*Klebsiella* spp.	*Salmonella* spp.	*Shigella* spp.	*Vibrio* spp.	*Pseudomonas* spp.	*Staphylococcus* spp.	*Bacillus* spp.	Fungi
Lara (n=5)	7×10^8	5×10^4	0	2.4×10^7	0	0	1.2×10^5	1.5×10^7	5.7×10^5	1.98×10^6
Quincy (n=5)	7.4×10^8	6×10^7	0	9.2×10^7	0	1.72×10^5	0	1.6×10^8	1.3×10^6	2.64×10^6
Cardinal (n=5)	5.4×10^8	4.3×10^5	0	0	5.2×10^6	0	1.3×10^5	7.5×10^5	0	8.2×10^5
Esprit (n=5)	9.8×10^8	6.3×10^5	0	0	7.7×10^6	0	1.3×10^7	9×10^5	0	7.3×10^5
Meridian (n=5)	8.7×10^8	7.7×10^5	0	6.3×10^6	0	2×10^5	5.4×10^6	7.3×10^5	0	8.5×10^5
Lady Rossett (n=5)	4.8×10^8	5.7×10^5	0	0	2.8×10^5	0	1.1×10^7	4.4×10^5	0	7.6×10^5
Astrix (n=5)	2.3×10^9	0	2.0×10^4	0	0	0	0	2.0×10^6	0	2.4×10^6
Soikat (n=5)	1.2×10^6	0	0	0	0	0	1.2×10^5	1.5×10^6	0	6.4×10^7
Yellow flesh (n=5)	7.5×10^7	5×10^4	0	0	1.3×10^6	0	0	1×10^7	0	1×10^5
Red (n=5)	2.5×10^8	0	0	0	8×10^5	0	0	2.8×10^5	0	2.8×10^6
Russet (n=5)	1×10^8	0	0	0	1.5×10^7	0	0	8×10^5	0	8×10^5
White (n=5)	2×10^7	4×10^5	0	0	7×10^5	0	0	1.3×10^5	0	1.3×10^7
Sweet (n=5)	2.5×10^7	2×10^5	0	0	-	0	0	2×10^7	0	2×10^7
Purples (n=5)	3.2×10^8	1.6×10^5	2×10^5	0	1.2×10^5	0	0	1.7×10^7	0	3.7×10^7
Petites (n=5)	1.2×10^7	0	0	1.4×10^5	0	0	0	2.6×10^7	0	2.8×10^7

TVB = Total Viable Count.
*Quantification was done "after enrichment". Before enrichment, the recovery was nil.
All the experiments were performed three times and the results were reproducible.

TABLE 2. Results of biochemical tests

Assumed Pathogenic microorganisms	TSI				Motility	Indole Production	MR	VP	Citrate utilization	Catalase	Oxidase
	Slant	Butt	Gas	H_2S							
Escherichia coli	Y	Y	+	-	+	+	+	-	-	+	-
Klebsiella spp.	Y	Y	+	-	+	-	-	+	+	+	-
Salmonella spp.	R	Y	-	+	+	-	+	-	-	+	-
Shigella spp.	R	Y	-	-	-	+	+	-	-	+	-
Vibrio spp.	R	Y	-	-	+	-	+	-	-	+	+
Pseudomonas spp.	R	R	-	-	-	-	-	-	-	+	-
Staphylococcus spp.	Y	Y	-	-	-	-	+	+	-	+	-
Bacillus spp.	R	Y	-	-	-	-	-	+	-	+	-

TSI = Triple Sugar Iron Test; Y = Yellow (Acidic), R = Red (Alkaline); MR = Methyl Red; VP = Voges-Proskauer

of the samples during growing, harvesting, storing and shipping. Food handlers with dirty hands or wearing soiled uniforms might also contaminate the items.

Existence of the drug-resistance isolates. In our study, most of the contaminating isolates were found to be resistant against ampicillin (10µg), amoxicillin (30µg), and erythromycin (15µg). Sensitivity was noted against ciprofloxacine (5µg), tetracycline (30µg) and chloramphenicol (30 µg) (Table 3). The resistance of microorganisms against more than one antibiotics mentioned in this study might pose public health hazards. Coherent with this study, previous studies conducted in Bangladesh on different food items and water also found huge array of drug resistant pathogens (16, 22, 28, 31, 34, 35). The resistance trait of the pathogens may create serious clinical obstacle in diseases medication during

TABLE 3. Antibacterial susceptibility test

Antibiotic	Disk content	*Klebsiella* spp.	*Salmonella* spp.	*Vibrio* spp.	*Staphylococcus* spp.	*E. coli*	*Shigella* spp.	*Bacillus* spp.
Polymyxin B	300 units	S	ND	ND	R	S	ND	S
Kanamycin	30 µg	ND	S	S	S	ND	ND	ND
Streptomycin	10 µg	ND	ND	ND	ND	ND	ND	R
Vancomycin	30 µg	ND	ND	R	ND	ND	R	S
Gentamicin	10 µg	ND	S	ND	ND	ND	S	S
Nalidixic acid	30 µg	ND	S	R	ND	ND	S	ND
Azythromycin	15 µg	ND	S	R	ND	ND	ND	ND
Penicillin G	10 µg	ND	ND	ND	R	ND	ND	R
Trimethoprime-Sulphamethoxazole	25 µg	S	S	ND	R	S	S	S
Erythromycin	15 µg	S	ND	ND	R	R	ND	S
Amoxicillin	30 µg	R	R	R	S	S	ND	S
Ceftriaxone	30 µg	R	ND	S	S	S	S	ND
Ciprofloxacin	5 µg	S	S	ND	S	S	S	ND
Streptomycin	10 µg	R	ND	S	S	R	ND	R
Ampicillin	10 µg	R	R	R	S	R	ND	S
Tetracyclin	30 µg	S	ND	R	S	S	ND	ND
Chloramphenicol	30 µg	S	S	R	S	S	S	R
Cefixime	5 µg	R	ND	ND	S	S	ND	S

R = Resistant; S = Susceptible; ND = Not done

food borne disease outbreaks.

Antibacterial profile of potato samples. Presence of any natural antimicrobial activity in such food items would be interesting as it could further induce the production of more effective drugs (39). Several studies assessed the natural antimicrobial activity of the vegetable samples globally (33, 34, 39, 40). The natural bacteria killing activity of the vegetables and/or other food samples could be a potential replacement of the chemical medicines, which can reduce the high possibility rate of the side effects of chemical medicine and can easily accelerate the shelf life of the vegetable. Based on those previous investigation, the present study determined the anti-microbial activity of the potato samples as a new research findings in Bangladesh (Table 4). Interestingly in present study, antibacterial activity was found in the Astrix samples (9 mm) against *Shigella* spp. and Russet samples (11 mm) against *Staphylococcus* spp. However, the other samples showed no antimicrobial activity against any of the bacterial isolates. Therefore, the findings suggest that further study could be conducted focusing the self-protecting mechanism of the potato samples.

CONCLUSION

Like other developed countries, people of Bangladesh are also leaning towards healthy and hygienic diets as the life style is changing. As suggested from other studies together with the current one, intake of vegetables contaminated with microorganisms may impart foodborne complications. Present study generated important data regarding food hygiene and service to different sectors of national economy such as agriculture, health, food and environmental sciences. Finally, the present work would further furnish important information about the sanitary condition of other vegetables that we consume and also on how we can minimize the risk of getting different diseases.

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