http://dx.doi.org/10.22161/ijeab/3.5.3 ISSN: 2456-1878

Prevalence of Microbial Loads on Betel Leaf with Special emphasis on Multidrug Resistance Salmonella spp and its Public Health Implications

Najmun Nahar*[1], Sapan Das[1], Abu Sufian[1], Md. Khairul Islam[1], Humaira Akter[2]

[1]Department of Microbiology, Gono Bishwabidyalay, Savar, Dhaka1344, Bangladesh.
[2]Departments of Microbiology, University of Dhaka, Dhaka 1000, Bangladesh.
*Corresponding authors email: dhipshikha@gmail.com

Abstract**—** *Presence of pathogen especially Salmonella spp in the Betel leaves suspended export of Betel leaf in Europe. Bangladesh has a subtropical monsoon so the present study was undertaken to determine Microbial loads of Betel leaf on the basis of seasonal variation (rainy and winter season). A total of 50 Betel leaf samples were collected from five sources (betel field, transport, whole seller, local shop, betel leaf washing water used in local shop Highest TVC (total viable count) were counted from local shop sample (5.3X 10^5CFU/ml) and the lowest TVC was found from field sample (2.5X 10^3 CFU/ml). This study results showed that during rainy season (July-October) TVC count was higher than winter season (November-February). From this study 10 genera of bacteria, were isolated from betel leaf such as E.coli, Vibrio spp, Bacillus spp ,Pseudomonas spp, Klebsiella spp, Salmonella spp, Shigella spp, Staphylococcus spp, Enterococcus spp and Proteus spp) and 5 genera of fungus (e.g. Aspergillus spp , Fusarium spp, Rhizopus spp, Zygosaccharomyces spp and Rhizoctonia spp)were isolated. Out of 184 isolates we found the following percentage of isolated microorganisms: 17.9% in betel leaf field, 19.5% in Transport, 19.5% in wholesaler, 28.8% in local shop and 14.3% in betel leaf washing water from local shop. Antibiotic sensitivity test showed that all of the isolates were resistant to Bacitracin, Penicillin, Vancomycin, Erythromycin and against other 5 antibiotics (Azithromycin, Gentamycin, Cephalexin, Ciprofloxacin and Chloramphenicol) isolates showed Resistant, Moderate and Sensitive Results.Data of this study suggest that Betel leaves from different source could harbor multidrug resistant bacteria specially Salmonella spp which underscore the need of implementation of hygienic practices during production, harvesting, transportation, storage, selling and preparation of Betel leaves to safeguard public health.*

Keywords**— Antibiotic Sensitivity, Betel leaf, Drug Resistance, Seasonal Variation, TVC.**

I. INTRODUCTION

Botanical name of betel vine is *Piper betel*. In Bangladesh, it is known as 'paan'. It is available in many Asian countries including Bangladesh. The betel plant originated from the South and South East Asia. The betel leaf is cultivated in most of South and Southeast Asia. Betel leaves has good export potential and Bangladesh exports betel leaves to the countries like Pakistan, India, Indonesia, Malaysia, Burma and Thailand. The harvested leaves are consumed locally or exported to other parts of Asia, the Middle East, Europe, and the United States.

In Bangladesh, farmer prepares a garden called a barouj in which they grow betel. The barouj is fenced with bamboo sticks and coconut leaves. The soil is plowed into furrows of 10 to 15 meters length, 75 centimeters in width and 75 centimeters' depth. Oil cakes, manure, and wood ash are thoroughly incorporated with the topsoil of the furrows. The creeper cuttings are planted at the beginning of the monsoon season. The harvest lasts for 15 days to one month. Betel plays an important role in the economy of rural Bangladesh. In some regions betel leaf cultivation is the main source of income for farmers. A total of 2,825 hectares of land is dedicated to betel vine farming. The average production cost for these betel farms in Bangladesh are about Tk. 300,000 per hectare, and the farm owners can earn a profit of over Tk. 100,000 per hectare. Betel vine is an important medicinal and recreational plant in Southeast Asia.

Betel leaf (Paan) export to the European and Middle Eastern countries stood at over US $ 31 million in 2012.

http://dx.doi.org/10.22161/ijeab/3.5.3 *ISSN: 2456-1878*

Detection of *Salmonella* bacteria in betel leaf from Bangladesh in the UK prompted the European Union to suspend imports. Expatriates from Bangladesh and India are the primary customers of betel leaf in European countries. Saudi Arabia and the USA are other big markets for betel leaf [1]. The government has taken an initiative to produce bacteria-free betel leaf in order to resume its export.

The surface of Betel leave can be contaminated with microbial pathogens by polluted air, water and soil, during pre-harvest stage. Packaging materials used for carry and storage at Betel leaf, moisture content and water used for washing of Betel leaf are important sources of contamination during post-harvest stage [2].

1.1 Rationality of the Study

According to the Food Standards Agency, UK- Since October 2011 there have been several food safety notifications concerning the presence of a range of pathogenic *Salmonella* strains found in foodstuffs containing or consisting of betel leaves originating in or consigned from Bangladesh. There is a temporary suspension of imports of betel leaves from Bangladesh until 30 June 2018 [3]. So it's high time to develop methods for controlling *Salmonella spp*. in the Betel leaf for local consumption.

Detection of *Salmonella* bacteria in betel leaf from Bangladesh in the UK prompted the European Union to suspend imports. Expatriates from Bangladesh and India are the primary customers of betel leaf in European countries. Saudi Arabia and the USA are other big markets for betel leaf [4].

The surface of Betel leaves can be contaminated with microbial pathogens by polluted air, water and soil, during pre-harvest stage. Packaging materials used to carry and storage Betel leaf, moisture content and water used for washing are important sources of contamination during post-harvest stage [5].

1.2 Prevalence of microorganism in food (leaf) products

Post- harvesting the spoilage of betel leaves accounts for the post-harvest loss in the range of 35%–75% respectively [6][7].

A comprehensive microbiological investigation of pathogen causing leaf diseases has been conducted to isolate, classify and characterize micro flora of Betel leaf. *Xanthomonas compestris* pv. *Betticola* bacteria have been identified previously from damaged Betel leaves [8].

Across Trinidad the prevalence and microbial load of *Listeria spp*. *Escherichia coli* O157 and *Salmonella spp*. was determined in the products of supermarkets. The microbial load was assessed using the total aerobic plate count (TAPC) per g/ml of foods and prevalence of *Escherichia coli* O157 and *Salmonella spp*. were determined using conventional methods. For *Listeria monocytogenes*, immune magnetic separation (IMS), TECRA (enzyme-linked immune sorbent assay, ELISA) and conventional methods were used. The $\log_{10}$ mean ± SD TAPC per g or ml was highest for vegetables (11.0±11.6), and lowest for seafood (5.2±5.7) ($p < 0.05$). The prevalence of *L. monocytogenes* was 1.7%. Sixteen (4.5%) of 153 samples yielded *E. coli* but all samples were negative for *Salmonella spp*. and *E. coli* O157 [9]

A significant bacterial count (CFU g) was detected in jhal-muri (1.66x10 CFU g), betel-leaf (1.49x10 CFU g), hog-plum (1.87x10 CFU g), sweet (3.39x10 CFU g) and bun (3.11x10 CFUg). Sola (6.24x10 CFU g), cup-cake (6.19x10 CFU g), peaju (4.96x10 CFU g), sheek-kabab (2.63x10 CFU g) an vhel-puri (1.96x10 CFU g) found to be contaminated with moderate bacterial count whereas, in singara (8.93x10 CFU g) and somosa (4.11x10 CFUg) load was found. Jhal-muri, hog-plum, betel-leaf, peaju, sheek-kabab, singara and vhel-puri were found to be 100 % contaminated with coliforms with an unacceptable range, as compared to somosa (75 %), sola (50 %) and bun (25 %). But cup-cake and sweet were free from contamination with coliforms (0 %). So among the 48 RTE food samples, 29.16 % of them did not contain coliforms. It was found that, sola (6596 CFU g), hog-plum (6197 CFU g), betel-leaf (3856 CFU g) and jhal-muri (2312 CFU g) were hazardously contaminated with fungi [10], when evaluated bacterial loads in salad vegetables using spread plate agar dilution method was done. Bacterial loads ranged from 1.6 x 10^6 to 2.9 x 10^8 CFU/g .*Escherichia coli*, *Klebsiella* and *Enterobacter* were amongst the Coliforms (lactose fermenters), while *Proteus*, *Pseudomonas aeroginosa*, *Salmonella* and *Shigella* were non-lactose fermenters associated with the samples.

Salmonella spp is an important zoonotic pathogen that cause an estimated 1.4 million illness, 16000 hospitalization and between 400 to 600 deaths annually in the united states alone [11][12]. *Salmonella* can produce invasive infections that lead to sepsis and death. Young children, the elderly and those with compromised immune systems are especially susceptible to severe disease.

The prevalence of multidrug resistant among *Salmonella* strain has increased over the past two decades [13][14][15], making treatment failures more common among those with serious disease. In addition, infections with resistant strains of Salmonella tend to be more severe and lead to higher rates of hospitalization than those caused by susceptible strains [16][17][18][19].And multidrug - resistant strains of zoonotic *Salmonella spp* present on ready-to-eat Paan is a public health concern. It may be one of the factors responsible for the hyper endemic status of salmonellosis [20]. People generally acquire salmonellosis through foodborne exposure,

http://dx.doi.org/10.22161/ijeab/3.5.3 *ISSN: 2456-1878*

although direct contact with infected animals is another possible route [21][22]. The outcome of different experiments showed that the best season for longer storage of betel leaves in any of the form which may be petiolated or depetiolated is winter seasons i.e. December-January [23] .

Fungus is any member of the group of eukaryotic organisms that includes microorganisms such as yeasts and molds as well as the more familiar mushrooms. These organisms are classified as a kingdom, Fungi which are separate from the other eukaryotic life kingdoms of plants and animals. Fungi spoiling organisms are silently invading, acidifying, fermenting, discolouring, and disintegrating microbes that render corn such as maize, wheat, barley etc. Fungi spoilage is caused by two factors, (biotic) living which includes insects, birds, rodents and microorganisms and (non-biotic) non-living which includes temperature, humidity and time. The world is concerned with food safety that has enhanced interest in fungal and subsequent food spoilage. Contamination with mould causes deterioration of product which affects human and animal health. [24]. Fungal spoilage of corn reduces the nutritional value and palatability of the feed, thereby increasing its allergic potential and may result in mycotoxic contamination [25].

II. RESULTS

2.1Collection and transportation of samples

A total of 50 betel leaf samples were collected from Different sources (betel field, transport, whole seller, local shop, betel leaf washing water used in local shop) on the basis of seasonal variation (rainy and winter season). Individual sample placed in the sterile container. The samples were transported carefully to the Bacteriology laboratory for bacteriological analysis.

2.2 Processing of betel leaf samples

The betel leaf samples in polythene-bag were washed with sterile PBS (phosphate buffered saline). One Betel leaf was washed with 20ml of sterile PBS. A 5 fold serial dilution of the washed samples was prepared in nutrient broth.

2.3 Determination of Total Viable count (TVC) of betel leaf

A total of 0.1 ml 10 fold diluted sample (10^{-1} to10^{-6}) was transferred and spreaded onto nutrient agar (NA) and incubated at 37ºC for 24-48 hours. TVC was determined by using the following formula

CFU/ml=Number of colonies/ml x dilution factor

Table.1: Total Viable count of betel leaf

Betel leaf source	Sample	Seasonal variation		TVC, CFU/ml
Route level(field)	1,6,11,16,21	Winter	1.68 X 10^4	1.82 X 10^4
	26,31,36,41,46	Rainy	1.96 X 10^4	
Transport	2,7,12,17,22	Winter	1.32 X 10^5	1.41 X 10^6
	27,32,37,42,47	Rainy	1.55 X 10^5	
Whole seller	3,8,13,18,23	Winter	2.7 X 10^5	2.64 X 10^6
	28,33,38,43,48	Rainy	2.58 X 10^5	
Local shop	4,9,14,19,24	Winter	4.42 10^4	2.31 X 10^6
	29,34,39,44,49	Rainy	4.18 X 10^5	
L.S.W.W	5,10,15,20,25	Winter	1.51x 10^5	1.98 X 10^6
	30,35,40,45,50	Rainy	2.46 X 10^5	

* L.S.W.W= Local Shop Washing Water

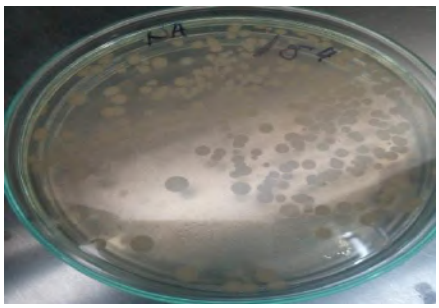

Fig1: Growth of microorganism on Nutrient Agar medium (TVC)

2.4 Statistical Data Analysis for significance level

Test for two independent Samples/Two –tailed test was performed to show statistical significance (Table-2).

A one way ANOVA (Table-3, 4) followed by Analysis of the differences between the categories Fisher (LSD) test (Table-5) were also used. We also conducted t Paired test, ANOVA test followed by Fisher (LSD) to find out whether our calculated value had any significance.

Table2: Test for two independent Samples/Two-tailed test, 95% confident interval on the difference between the means of different collection site

http://dx.doi.org/10.22161/ijeab/3.5.3 *ISSN: 2456-1878*

Variable	Rainy	Winter
Observations	25	25
Obs. with missing data	0	0
Obs. without missing data	25	25
Minimum	10000	10000
Maximum	630000	300000
Mean	219440	122960
Std. deviation	187260.754	117572.488
Difference		96480
t (Observed value)		2.182
|t| (Critical value)		2.011
DF		48
p-value (Two-tailed)		0.034
Alpha		0.05

t-Paired test interpretation

H0: The difference between the means is equal to 0.

Ha: The difference between the means is different from 0.

As the computed p-value is lower than the significance level alpha=0.05, we can reject the null hypothesis H0, and accept the alternative hypothesis Ha

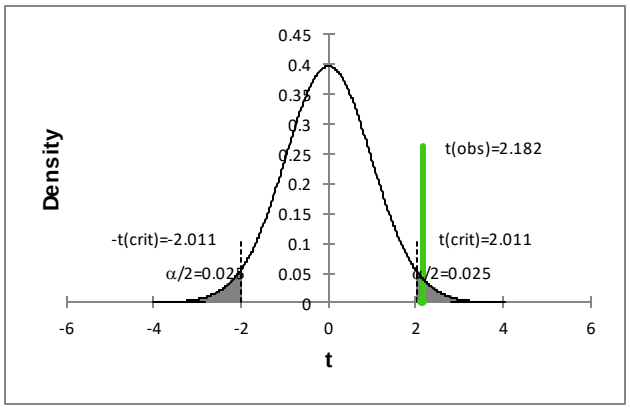

Fig2: t-test for two independent samples / Two-tailed test

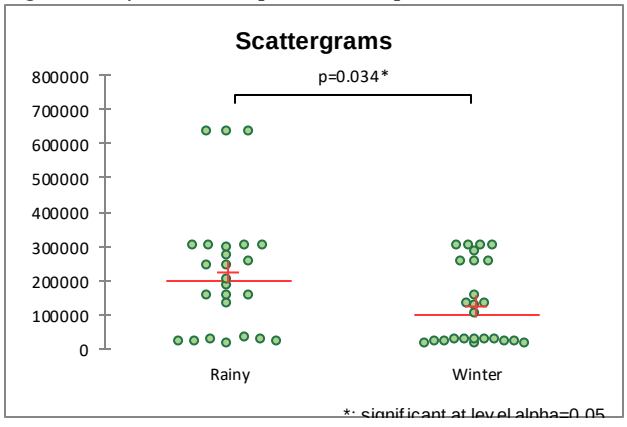

Fig 3: Scatter grams of TVC Count on the basis of Rainy and Winter Season.

Table 3: Summary statistics (Quantitative data): One way ANOVA test

Variable	Observations	Obs. with missing data	Obs. without missing data	Minimum	Maximum	Mean	Std. deviation
TVC	50	0	50	10000	630000	171200	162236.411

Table 4: Analysis of variance, ANOVA (TVC)

Source	DF	Sum of Square	Mean square	F	Pr>F	F Crit
Between Groups	4	370940200000.000	92735050000.000	4.542	**0.004**	**2.45**
Within Groups	45	918771799999.999	20417151111.111			
Total	49	1289712000000.000				

Our Calculated value, F (4, 45) = 4.452, P=0.004 is higher than F Critical Value 2.45 so there is a significant difference among the TVC count of different collection Site.

http://dx.doi.org/10.22161/ijeab/3.5.3 *ISSN: 2456-1878*

Table 5: Summary of all pair wise comparisons for C.S (Fisher LSD)

Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups	
Wholeseller	264000	45185.342	172992.050	355007.950	A	
Local shop	231100	45185.342	140092.050	322107.950	A	
L.S.W.W	198600	45185.342	107592.050	289607.950	A	
Transport	144100	45185.342	53092.050	235107.950	A	B
Field	18200	45185.342	72807.950	109207.950		B

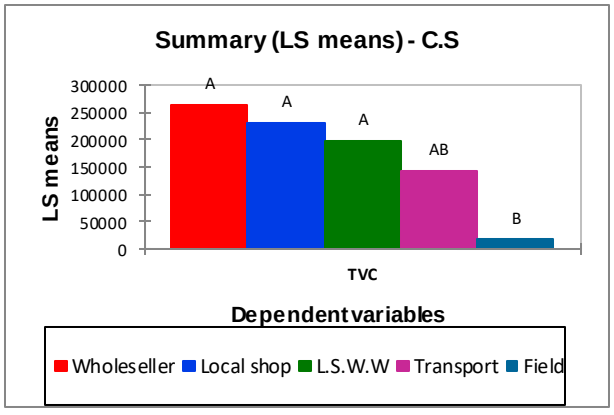

Fig 4: Bar diagram of TVC Count (LS means) on the basis of Collection Site.

2.5 Isolation and Identification of Microorganisms

After Microscopic observation followed by cultural and biochemical test results observation , 10 genera of bacteria (e.g. *E.coli*-21.73%, *Vibrio* spp - 7.6% ,*Bacillus spp* -2.7%, *Pseudomonas* spp-3.84%, *Klebsiella* spp - *7.06%* , *Salmonella* spp -19.5%, *Shigella* spp - 5.43%,*Staphylococcus* spp-5.43%, *Enterococcus* spp-*4.89%* and *Proteus* spp-*1.63%*) and 5 genera of fungus (e.g. *Aspergillus* spp-*5.43%*, *Fusarium* spp- *4.89%*. *Rhizopus* spp-*3.84%*, *Zygosaccharomyces* spp- *3.26%*, *Rhizoctonia* spp-*2.71%*) were isolated [26] [27].

Out of 184 isolates we found the following percentage of isolated microorganisms 17.9% in betel leaf field, 19.5% in Transport, 19.5% in wholesaler, 28.8% in local shop and 14.3% in betel leaf washing water from local shop.

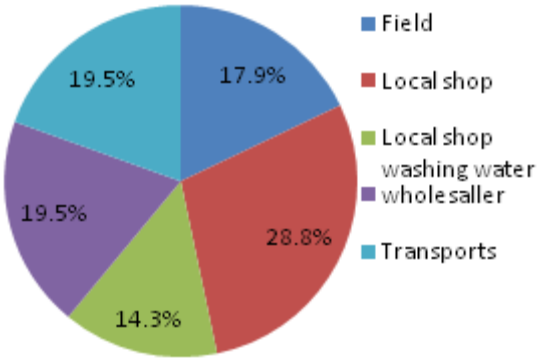

Fig.5: Percentage of isolated pathogen from betel leaf

Antibiotic sensitivity test showed that all of the isolates were resistant to Bacitracin , Penicillin, Vancomycin, Erythromycin and against other 5 antibiotics (Azithromycin, Gentamycin, Cephalexin , Ciprofloxacin and Chloramphenicol) isolates showed Resistant, Moderate and Sensitive Results. [28]

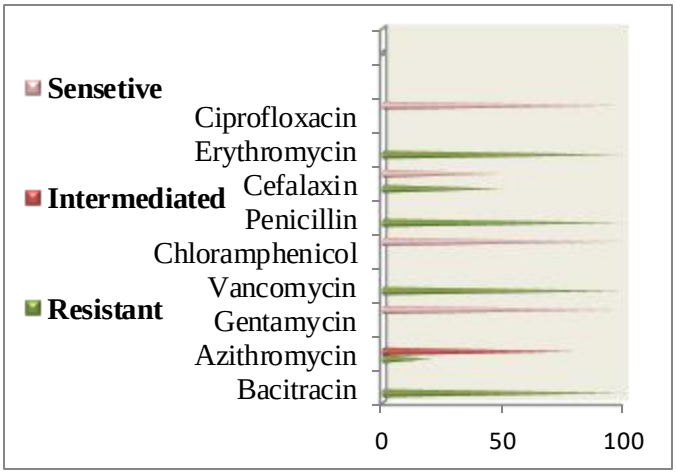

Fig.6: Antibiotic Sensitivity of Bacteria isolated (total 125) from betel leaf

http://dx.doi.org/10.22161/ijeab/3.5.3 *ISSN: 2456-1878*

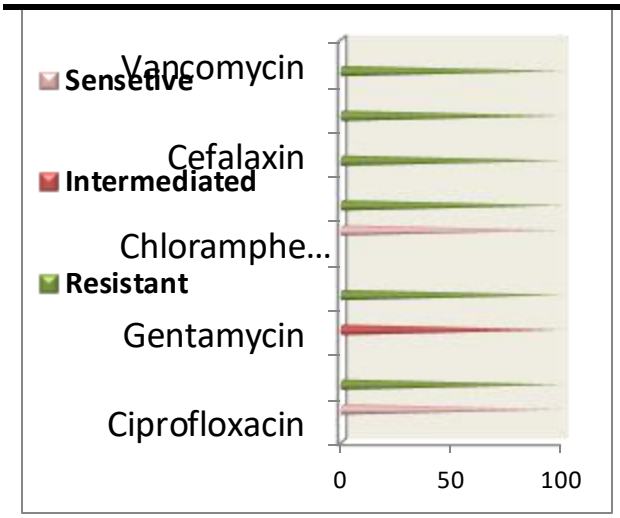

Fig.7: Summary of antibiogram profile of Salmonella spp. against 9 antibiotics.

III. DISCUSSION

Highest TVC counted from Whole seller sample (2.64 X 10^6) and the lowest TVC counted from field sample(1.82 X 10^4).TVC count from different source vary might be due to unsanitary environment, use of polluted water to wash Betel leaf and unclean utensil used to storage Betel leaf.

This study results showed that during rainy season (July-October) TVC count was higher than winter season (November-February).

We also conducted t Paired test, ANOVA test followed by Fisher (LSD) to find out whether our calculated value had any significance. From the statistical data analysis we have found that our observed data were significant at 95% confidence level.

In the present study, selective media (EMB, TCBS. SS. Macconkey, SDA, MSA) were used for isolation of *E. coli, Vibrio* spp. *Salmonella* spp.,and *Klebsiella spp Bacillus spp, Staphylococcus spp, Enterococcus spp* .

In this study, the colony characteristics of *Vibrio* spp. on TCBS agar plate were similar to the findings of Tankeshwar Acharya. In Gram's staining bacteria exhibited curved rod shaped appearance which was also observed by other researchers [29][30][31].

The colonies of *Salmonella* spp. on agar SS plate were opaque, translucent with black centers which were similar to the findings of Cheesbrough.[32].In Gram's staining *Salmonella* spp. exhibited short rods, Gram negative, single or paired in arrangement. Similar findings were also reported by Buxton and Frase [33][34].

From this study 10 genera of bacteria, were isolated from betel leaf such as *E.coli*-21.73%, *Vibrio spp -7.6%, Bacillus* spp -2.7%, *Pseudomonas* spp-3.84%, *Klebsiella* spp-*7.06%* , *Salmonella* spp-19.5%, *Shigella* spp-5.43%,*Staphylococcus* spp-5.43%, *Enterococcus* spp-*4.89%* and *Proteus* spp-*1.63%*) .

A study conducted in Bangladesh found that the prevalence of *E. coli* was 17.34% (17 of 98), *Salmonella* spp. was 25.51% (25 of 98), *Vibrio* spp. was 19.39% (19 of 98), *Bacillus* spp. was 18.37% (18 of 98), and *Staphylococcus* spp. was 19.39 (19 of 98) [35].

A total of 5 genera of fungus (e.g. *Aspergillus spp-5.43%, Fusarium spp- 4.89%. Rhizopus spp-3.84%, Zygosaccharomyces spp- 3.26%, Rhizoctonia spp-2.71%*) were isolated

A study conducted in India isolated from Betel leaves isolated *Xanthomonas compestris* PV. Betticola fungi from diseased Betel leaves. **[2]**

From our observation out of 184 isolates we found the following percentage of isolated microorganisms: 17.9% in betel leaf field, 19.5% in Transport, 19.5% in wholesaler, 28.8% in local shop and 14.3% in betel leaf washing water from local shop.

In case of *Salmonella* we have found that 38% of betel leaf sample was contaminated with *Salmonella* spp. Among them 7, 6 and 5 no of *Salmonella spp* were isolated from Transport, Whole seller and Local Shop Betel leaf Samples respectively. Our study showed that Transport is the major source of *Salmonella* spp contamination in Betel leaf consumed in Bangladesh.

A study conducted in Bangladesh found that77% betel leaf sample collected from different markets of Dhaka city was found to be contaminated with Salmonella spp. [36].

Antibiotic sensitivity test show most of the isolates were resistant to bacitracin, penicillin. More shocking report is, most of the people in Bangladesh use Erythromycin and Azithromycin antibiotic vigorously but this study show erythromycin were resistant against four isolates and azithromycin show both moderate and resistant result and Ciprofloxacin was sensitive to all tested isolates. On the other hand gentamicin shows sensitive against the isolates. Cephalexin show both sensitive and resistant result [37].

Our isolated 18 isolates of *Salmonella spp* showed completely resistance to Bacitracin, Penicillin, Vancomycin, Erythromycin, Azithromycin, Amoxicillin and sensitive against other 2 antibiotics, Ciprofloxacin and Chloramphenicol. They are intermediately sensitive to Amoxicillin. Indiscriminate use of antibiotic is responsible for emergence of multidrug resistant *Salmonella spp*. [19] [38].

Data of this study suggest that Betel leaves from different source harbor multidrug resistant [39] bacteria which underscore the need of implementation of hygienic practices during production, harvesting, transportation, storage, selling and preparation of Betel leave to safeguard public health. From this study we could suggest that Betel leaves might be contaminated with bacteria not

only due to use of potable water for washing, handling of Betel leave with unclean hands but also use of unclean utensil or cutting board when preparing ready to eat Betel leaves.

IV. CONCLUSION

Data of this study suggest that Betel leaves from different source harbor multidrug resistant bacteria which underscore the need of implementation of hygienic practices during production, harvesting, transportation, storage, selling and preparation of Betel leaves to safeguard public health.

ACKNOWLEDGEMENTS

The authors would like to thank the Research Cell, Gono Bishwabidyalay, Savar Dhaka for funding the project. The authors also acknowledge the University of Dhaka for their laboratory support for a part of this study.

REFERENCES

[1] Chowdhury, K.R. and Kallol, A.S. (2013) EC Warns Bangladesh of Illegal Betel Leaves Export. Dhaka Tribune. Available from: http://www.dhakatribune.com/business/ commerce/2014/02/05/ec-warns-bangladesh-of-illegal-betel-leaves-export/ Accessed on 06-02-2017.

[2] Council of Scientific and Industrial research (CSIR) Wealth of India,1984,8:87.

[3] EUR-Lex 2016.Amending Implementing Decision 2014/88/EU suspending temporarily imports from Bangladesh of foodstuffs containing or consisting of betel leaves ('Piper Betle') as regards its period of applicatio .Available from: https://eur-lex.europa.eu/legalcontent/EN/TXT/?uri=CELEX%3A32016D0884 Accessed on 18-08-2017

[4] P. Guha .Betel leaf: the neglected green gold of India J Hum Ecol, 19 (2) (2006), pp. 87-93

[5] Sharada,R.,Ruban, s.w and thiyageeswaran,m.2010 Isolation and characterization of Ecoli from pultry and their antibiogram.American-Eurasian j. Sci,Res.5(1):18-22

[6] V. Rao, B. Narasimhan. Prolonging storage of betel leaves Ind J Hort, 34 (1997), pp. 175-182

[7] H.Mohanta. , A.Pariari,2012 Effect of climatic factors on the growth and leaf yield of betelvine (*Piper betle* L.) *Journal of Applied and Natural Science* 8 (2): 1006 - 1009 (2016)

[8] Mishra V., and Mishra A ,.A study of Betel leaf microflora.Asian Journal of Experimental Science, 200519: 59-62.

[9] Hossain KM, 2002. Characterization of bacteria isolated from diarrhoeic calves. M.S. Thesis, Department of Microbiology and Hygiene, Faculty of Veterinary Science, BAU, Mymensingh,pp. 45-53.

[10] Mercy AI, Peters O, Kolo I 2011: Bacterial contaminants of salad vegetables in Abuja Municipal Area Council, Nigeria. Malaysian Journal of Microbiology, 7:111-114.

[11] Mead PS., Slutsker L., Dietz V., McCaig LF., Bresee JS., Shapiro C., Griffin PM., and Tauxe RV.Food-related illness and death in United States. Emerge Infect Dis.1999;5:607-625.

[12] Voetsch AC., Van Gilder TJ., Angulo FJ., Farley MM., Shallow S., Marcus R., Cieslak PR., Deneen VC. and Tauxe RV. Food Net estimate of the burden of illness caused by nontyphoidal Salmonella infections in the United States. Cin Infect Dis.2004;38(Supp 3): S127-S134.

[13] Glynn MK., Bopp C., Dewitt W., Dabney P., Mokhtar M., and Angulo FJ. Emergence of Multidrug-resistant *Salmonella enterica* seroype *Typhimurium* DT104 infections in theUnited States. N Engl J Med.1998;338:1333-1338.

[14] Dunne EF, Fey PD, Kludt P, Reporter R, Mostashari F, Shillam P, Wicklund J, Miller C, Holland B, Stamey K, Barrett TJ, Rasheed JK, Tenover FC, Ribot EM and Angulo FJ. Emergence of domstrically acquired ceftriaxone-resistant *Salmonella* infections associated with AmpC beta-lactamase. JAMA. , 2000;284:3151-3156.

[15] Davis, M.A., Besser, T.E., Eckmann, K., Macdonald K., Green D., Hancock D.D., Baker K.N., Warnick L.D., Soyer,Y., Wiedmann, M., and Call D.R. . Multidrug-resistant Salmonella Typhimurium, Pacific Northwest, United States. *Emerg. Infect. Dis.*, 2007 ;13:1583–1586.

[16] Helms M. , Vastrup P. , Gerner-Smidt P. , and Molbak K,. Excess mortality associated with antimicobial drug-resistant *Salmonella Typhimurium*. Emerge Infect Dis. 2002;8:490-495.

[17] Helms M. Simonsen J. and Mobak K.. Quinolone resistance is associated with increased risk of invasive illness or death during infection with *Salmonella* serotype *Typhimurium* Infect Dis. , 2004;190:1652-1654 .

[18] Varma JK., Greene KD., Ovitt J., Barrett TJ., Medalla F., *and* Angulo FJ. Hospitalization and antimicrobial resistance in Salmonella outbreaks, 1984–2002. Emerg Infect Dis. 2005a;11: 943–946.

[19] Varma JK., Molbak K., Barrett TJ., Beebe JL., Jones TF., Rabatsky-Ehr T., Smith KE., Vugia DJ., Chang HG., Angulo FJ. Antimicrobial-resistant nontyphoidal Salmonella is associated with excess bloodstream infections and hospitalizations. J Infect Dis. 2005b; 191: 554–561.

[20] SinghBR,SinghM,SinghP,BabuN,ChandraM,AgarwalRK,2006. Prevalence of multidrug-resistant Salmonella on ready-to-eat betel leaves (Paan) and in water used for soaking betel leaves in North Indian Cities. Journal of FoodProtection,69:288-292.

[21] L Plym and Wierup M. Salmonella contamination. A significant challenge to the global marketing of animal food products. Rev Sci Tech. 2006;25:541-554.

[22] Hosein, A., K. Munoz, K. Sawh and A. Adesiyun, 2008. Microbial load and the Prevalence of Escherichia coli, Salmonella spp. and Listeria spp. in ready-to-eat products in Trinidad. The Open Food Science Journal, 2: 23-28

[23] M.N. Imam, A. PariariKeeping quality of betel leaves (*Piper betle* L.) as influenced by different methods and seasons J Spices Aromat Crops, 21 (2) (2012), pp. 106-112

[24] Afsana Jahan (December, 2013) Investigation On The Leaf Rot And Foot And Root Rot Of Betel Vine (Piper Betle L.) In Satkhira District Of Bangladesh. M.S. Thesis. Department Of Plant Pathology Sher-E-Bangla Agricultural University Dhaka-1207

[25] The independent,dhaka,6 may 2011. Betel leaf farming benefiting farmers

[26] Bergey's manual of determinative bacteriology 2000

[27] Bhagra S, Kanga A, Ganju SA, Sood A. Antibiotic susceptibility pattern of Salmonella enterica serovar Typhi and Paratyphi a from North India:The changing scenario. Int J Pharm Bio Sci. 2014;5(4):1–9.

[28] Bauer, A.W., Kirby, W.M.M., Shrris, J.C. and Truck, M. 1966. Antibiotic susceptibility testing by a standardized single disc method. Am. J. Clin. Pathol., 45: 493-496. [PubMed]

[29] Rao.Sridhar. Vibrio cholerae: cholera applied microbiology cases <web.www.cdc.gov/cholera/index.html, December04, 2010

[30] Khan, A.W., Hossain, S.J. and Uddin, S.N. (2007) Isolation, identification and determination of antibiotics susceptibility of *Vibrio parahaemolyticus* from shrimps at Khulna region of Bangladesh. *Res. J. Microbiol.,* 2: 216-227.

[31] Kaper JB, Morris JG, Levine MM 1995. Cholera. Clinical Microbiology Review, 8: 48-86.

[32] Cheesbrough M, 1985. Medical laboratory manual for tropical countries. 1st edn. Microbiology. English Language Book Society, London,pp. 400-480.

[33] Buxton A and Fraser G 1977. Animal Microbiology. Blackwell Scientific Publications, Oxford, London, Edinburg, Melbourne, pp. 400-480.

[34] Merchant, I.A. and Packer, R.A. (1967) The genus *Salmonella*. In: Veterinary Bacteriology and Virology. 7th ed., Vol. 43. Iowa State University Press, Ames. Iowa, USA. p286-306.

[35] Hoque. A., Khatun. A., Shah F. Mohammad, M. Asim, M. Tariq, K. A. Zaman and Hussain ., Faruquee. Md. Microbiological Hazard Analysis and Exposure Assessment of Street Vended Ready-to-Eat Foods in Dhaka City, Bangladesh. American-Eurasian J. Agric. & Environ. Sci.,, 2015.,15 (9): 1725-1731.

[36] Fakruddin, M., Sultana, R., Hossain, M.N., Rahaman, M.M., Kamrul, M.I. and Ahmed, M.M. (2017) Occurrence of ingression of *Salmonella* spp. In betel leaf (*Piper betle* L.). *Int. J. Food. Contam.*, 4: 1-10.

[37] Capoor MR, Nair D. Quinolone and cephalosporin resistance in enteric fever. J Glob Infect Dis. 2010;2(3):258–62. [PMC free article] [PubMed]

[38] S, Jain S, Prasad KN, Ghoshal U, Dhole TN. Rationale of azithromycin prescribing practices for enteric fever in India. Indian J Med Microbiol. 2012;30(1):30–33. [PubMed]

[39] Livermore DM., 2004 Livermore DM. The need for new antibiotics. Clin. Microbiol. Infect. 2004;10(Suppl 4):1–9. [PubMed]