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Antibiotic Sensitivity Pattern of Bacteria Isolated From Infected Betel Leaf

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Abstract: *The research work was carried out for detection of antibiotic sensitivity pattern of bacteria isolated from infected betel leaf sold at local markets of Dinajpur city, Bangladesh. Total 20 infected betel vine sample were collected from five different local markets (Leilimorr, Terminal road, Doshmile, Gopalgong bazaar, and Basharat) of Dinajpur. A series of test were conducted for isolation, identification and frequency distribution of different bacteria of betel leaf. A total of 20 bacterial isolates belong to five genera (staphylococcus spp., Bacillus spp., Escherichia coli, klebsiella spp. and Enterobacter spp.) were identified. Out of 20 samples, 6 were Staphylococcus spp. (30%), 4 were Bacillus spp. (20%), 1 were Escherichia coli (5%), 5 were klebsiella spp. (25%) and 4 were Enterobacter spp. (20%). The identified isolation was subjected to antibiogram study in which Staphylococcus spp. (6) were sensitive to Gentamicin (100%), followed by ciprofloxacin (83.33%), Vancomycin (66.66%), Erythromycin (33.33%), and resistant to Kanamycin (83.33%), Bacillus spp. (4) were found sensitive to Erythromycin (100%), were sensitive to followed by ciprofloxacin (75%), Neomycin (75%), Co-trimoxazole (50%), and resistant to Amoxicillin (100%). E.coli spp. (1), were found sensitive to Ciprofloxacin (100%), Co-trimoxazole (100%), Neomycin (100%). and resistant to Erythromycin (100%), and Amoxicillin (100%). klebsiella spp. (5), were found sensitive to Ciprofloxacin (100%), were sensitive to followed by Co-trimoxazole (80%), Neomycin (80%), and resistant to Amoxicillin (100%), Erythromycin (100%). Enterobacter spp. (4), were found sensitive to ciprofloxacin (100%), were sensitive to followed by kanamycin (75%), and resistant to Amoxicillin (100%), Ampicillin (100%), and Cefuroxime Sodium (100%). So Antibiogram result indicated the ciprofloxacin, Co-trimoxazole and Gentamycin, in optimum doses would be the drug of choice to treat the most cases of human infection caused by consumption of infected betel leafs.*

Keywords: *Antibiogram, Antibiotic sensitivity, Betel leaf, Bacteria identification.*

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I. Introduction

The deep green heart shaped leaves of betel vine are popularly known as Paan in Bangladesh also an important cash crop in Bangladesh. It is also known as Nagaballi, Nagurvel, Saptaseera, Sompatra, Tamalapaku, Tambul, Tambuli, VakshaPatra, Vettilai, Voojangalataetc in different parts of India (CSIR, 1969; Guha and Jain, 1997). The scientific name of betel vine is *Piper betle* L. under the family Piperaceae. It is a climbing plant with shiny, green heart-shaped leaves. Among about 100 varieties of betel vine in the world, of which nearly 40 are found in India and 30 in West Bengal (Guha, 1997; Maity, 1989; Samanta, 1994).Desi Bangla, Bangla, Kali Bangla, Jhali, Sanchi, Bhabna, Mitha, Geso, Bonhoogly etc. betel vine cultivars are found in Bangladesh. The most probable place of origin of betel vine is Malaysia (Chattopadhyay and Maity, 1967). Bangladesh is the second largest grower of betel vine on about 14,000 hectares. Total annual production of the crop in Bangladesh is about 72,500 tons. The average yielding rate per acre is 2.27 tons (Anonymous, 2006).

All classes of people in Bangladesh chew betel vine not only as a habit but also as an item of rituals, etiquette and manners. where leaves are traditionally used for chewing in their natural raw condition along with many other ingredients like sliced areca nut, slaked lime, coriander, aniseed, clove, cardamon, sweetener, coconut scrapings, ashes of diamond, pearl, gold and silver (Ayurvedic preparations), jelly, pepper mint, flavouring agent, fruit pulp etc. (CSIR, 1969). Disease damage to the crop is one of several known limiting factors. The betel vine is highly susceptible to diseases, pests and some natural climates (Sayeeduzzaman, 1988).

A number of leaf spot diseases have been reported of which that due to *Colletotriehum* is most important (Maiti and Sen, 1979). It causes both leaf spot and stem anthracnose. Although bacterial pathogens have been reported, bacterial leaf spot is now considered to be caused by *Xanthomonascampestrispv. betlieola*. Although much emphasis has been given to foot rot diseases, leaf spot diseases are also important as leaves are the commercial produce and a small spot on the leaf can reduce its marketability to a great extent. Leaf spot

caused by *Xanthomonascampestrispv. betlicola* has much similarity to that caused by *Colletotrichum.* In leaf spot caused by *Colletotrichum,* lesions are brownish black surrounded by yellow halo. On stem, elongated dark brown lesions are formed. The only difference with the bacterial disease is the water soaked slimy band on the advancing margin of lesion detected on lower surface of leaf. In stem lesion no apparent difference except that surface of stem lesion caused by bacterial pathogen is somewhat slimy. Because of this similarity, earlier also the bacterial leaf spot was 1U existence but was ignored due to similarity of symptom with that of *Colletotrichum* leaf spot. Wilting of betelvine plants ill different states of India is considered to be caused by *Phytophthora* or *Sclerotium sp*. but as the leaf spot bacteria also infect stem, it might have a role in wilt complex of betelvine. At present farmer's view in West Bengal is that wilt of vine is more severe than earlier days and symptom of diseases is different from that previously observed (Bidhan Chandra KrishiViswavidyalaya 2002).

Considering the importance of betel vine as a commercial crop in Bangladesh and also considering the public health importance. Therefore, the present study was designed i) To isolate and identify some important bacteria from betel leafii) To detect the best antibiotic sensitivity pattern of the isolated bacteria from betel leaf.

II. Materials and Methods

The present research work was conducted in the Bacteriology Laboratory of Department of Microbiology, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh science & Technology University (HSTU), Dinajpur. The experimental work was divided into two steps: the first step was performed for the isolation and identification of the organisms of the collected sample using cultural, staining and biochemical techniques. The second step was conducted for the determination of antibiotic sensitivity and resistance pattern of isolated organisms by using different antibiotic discs available in the market.

2.1 Collection and Preparation of Sample

First, Total 20 number of the betel leaf samples were collected from different markets located in, SadarUpozila, Dinajpur, (Leilimorr, Terminal road, Doshmile, Gopalgong bazaar, and Basharat).Then Brought the sample to the laboratory sealed poly bags to prevent their contact with any other source that by maintaining aseptic condition. After that, the sample was grinded by mortar and pestle & processing of the sample (dilution with PBS 1:9).

2.2isolation and identification of the organisms

Cultural test on Nutrient broth and Nutrient agar and Gram's staining performed respectively. For Gram positive bacteria cultured on Mannitol salt agar and blood agar then Gram staining and India ink satin performed. If Gram Negative Sub-cultured onto Mac Conkey agar (MAC) &then Sub-cultured on Eosin Methyl Blue agar (EMB) respectively, also Gram's staining performed. Lastly, Biochemical characterization of isolates using Indole, Citrate, TSI, MR-VP and MIU. Incubate at 37c 24, 48 and 72 hours and Spreading of pure bacterial culture colony into Mueller Hinton agar then Determination of antibiotic sensitivity of bacterial isolates against ten common antibiotics by Disc-diffusion method.

2.3 Antibiotic susceptibility test

Bacterial susceptibility to anti-microbial agent was determined in vitro by using the standardized agar disc-diffusion method known as the *Kirby Bauer*, Labeled the covers of each of the agar plates with name of the test organisms was inoculated.

- Using sterile technique, inoculated all agar plates with their respective test organisms as follow:
  - Dipped a sterile cotton swab into a well-mixed saline test culture and removed excess inoculums by pressing the saturated swab against the inner wall of the culture tube.
  - Using the swab streaked the entire agar surface horizontally, vertically, and around the outer edge of the plate to ensure a heavy growth over the entire surface.
- Allowed all culture plates to dry for about 5 minutes.
- Distributed the individual antibiotic discs at equal distance with forceps dipped in alcohol and flamed.
- Gently pressed each disc down with the wooden end of the cotton swab or sterile forceps to ensure that the discs adhered to the surface of the agar.
- The plates were then inverted and incubated at 37^0 C for 24 hours.
- After incubation, the plates were examined and the diameter of the zones of complete inhibition was measured in mm.
-

III. Results And Discussion

Total 20 numbers of infected betel leaf samples were collected for this study. Out of 20 samples 20 bacterial isolates were found, among them 6 were *staphylococcus spp*, 4 were *bacillus spp*., 4 were *Enterobacter spp*., and 5 were *klebshella spp.* And 1 *were E. coli spp.*The prevalence of bacterial isolates in betel leafs are diagrammatically illustrated in the pie chart,figure 1.

Cultural, morphological and biochemical properties of isolated *staphylococcus spp., Bacillus ssp., Escherichia coli, klebsiella & Enterobacter spp.* are shown in Table 2, Table 3, Table 4, Table 5, Table 6 respectively. Cultural and morphological characteristics of bacterial isolates are illustrated in the figure 2. and Figure 3. Respectively.

3.1 Result of antibiotic sensitivity pattern of isolated bacteria

3.1.1 Antibiotic sensitivity pattern of *Staphylococcus spp.* (n=6)

The antibiotic study revealed that all of isolates (6) were sensitive to Gentamicin (100%), followed by ciprofloxacin (83.33%), Vancomycin (66.66%), Erythromycin (33.33%) and the isolates were found resistant Kanamycin (83.33%). Shown in the table 7 and Figure 4.

3.1.2 Antibiotic sensitivity pattern of *Bacillus spp.* (n=4)

The antibiotic study revealed that all of isolates (4)were sensitive to Erythromycin 100% were sensitive to followed by ciprofloxacin (75%), Neomycin (75%), Co-trimoxazole (50%) and the isolates were found resistant Amoxicillin (100%).Given in the table 8 and Figure 5.

3.1.3 Antibiotic sensitivity pattern of *E. coli spp.* (n=1)

The antibiotic study revealed that all of isolates (1)were sensitive to Ciprofloxacin, Co-trimoxazole (100%), Neomycin. (100%). And the isolates were found resistant Erythromycin (100%). and Amoxicillin (100%).Presented in the table 9 and Figure 7.

3.1.4 Antibiotic sensitivity pattern *of Klebsiella spp.* (n=5)

The antibiotic study revealed that all of isolates (5), were found sensitive to Ciprofloxacin (100%) were sensitive to followed by Co-trimoxazole (80%), Neomycin (80%). And the isolates were found resistant Amoxicillin (100%), Erythromycin (100%).Given in the table 10 and Figure 6.

3.1.5 Antibiotic sensitivity pattern of *Enterobacter spp.* (n=4)

The antibiotic study revealed that all isolates (4)were sensitive to ciprofloxacin (100%) were sensitive to followed by kanamycin (75%). and the isolates were found resistant Amoxicillin (100%), Ampicillin (100%), Cefuroxime Sodium (100%). Shown in the table 11 and Figure 8.

3.2 Discussion

The experiment was carried out to isolate and identify bacteria as well as to detect antibiotic sensitivity pattern of isolated bacteria from infected betel leaf sold at local markets of Dinajpur city, Bangladesh. For this study, a total 20 of infected betel vine sample were collected from five different local markets (Leili mall, Terminal road, Doshmile, Gopalgong bazaar, and Basharat) of Dinajpur. A series of test were conducted for isolation, identification and frequency distribution of different bacteria of betel leaf. A total of 20 bacterial isolates belong to five genera (*staphylococcus spp., Bacillus, spp., Escherichia coli, klebsiella spp and Enterobacter spp*.) were identified. The prevalence of *staphylococcus spp. was* 30%, *Bacillus spp.* was 20%, *Escherichia coli* was 5%, *klebsiella spp*. was 25% and *Enterobacter spp*. was 20%. These findings were more or less similar to the findings made by Md. Mazedul Haque *et al.* (2017), in which they reported the prevalence of *Escherichia coli, Bacillus spp*. and *Staphylococcus spp.* inbetel leaf samples was 17.34%, 18.37%, and 19.39% respectively. Many infectious agents could be implicated as causes of betel leaf contamination but *Staphylococcus* spp, *Bacillus* spp, *E. colispp*, *Klebsiella spp*, *Enterobacter spp*, were isolated from infected betel leafs in this study. The frequency distribution of different species bacteria isolates in different betel leafs sample were found variable. Result of this study indicated that all the five different types of bacteria were not present in the same betel leaf sample collected from the different markets. *Staphylococcus spp* have been reported as the main pathogen of infected betel leafs. The *staphylococcus spp., bacillus spp., E. coli, klebsiella spp.* and *Enterobacter spp.* showed identical result in different biochemical test of including catalase test, indole test, methyl-red, voges-Proskauer, motility indole urease test, triple sugar iron test citrate utilization test.

The in vitro antibiotic sensitivity test of isolated bacteria 10 different antibiotics such as Gentamycin, Ciprofloxacin, Cefuroxime sodium, Co-trimoxazole, Kanamycin, Neomycin, Erythromycin, Amoxicillin, Vancomycin, and Ampicillin ware used. Staphylococcus was study a major variation was noticed in the result of sensitivity of isolation agent's 5 different antibiotics use.

From the antibiotic sensitivity test of *staphylococcus spp.*, it was found that 100% of the isolated *staphylococcus spp.* was sensitive to Gentamicin followed by ciprofloxacin (83.33%) Vancomycin (66.66%), Erythromycin (33.33%) and Amoxicillin (16.66%).These findings were more or less similar to the findings of Md. Mazedul Haque *et al.* (2017).

From the antibiotic sensitivity test *Bacillus spp*., it was found that 100% of the isolated *Bacillus spp.* was sensitive to Erythromycin followed by ciprofloxacin (75%), Neomycin (75%) Co-trimoxazole (50%) and Amoxicillin (25%). These findings were more or less similar to the findings of Md. MazedulHaque *et al.* (2017).
From the antibiotic sensitivity test *E. coli*, it was found that 100% of the isolated *E.coli* was sensitive to Ciprofloxacin, Co-trimoxazole, Neomycin. 100% resistant against Erythromycin and Amoxicillin. These findings were more or less similar to the findings of Md. Mazedul Haque *et al.* (2017).
From the antibiotic sensitivity test *Klebsiella ssp*,it was found that 100% of the isolated *Klebsiella spp.* was sensitive to Ciprofloxacin followed by Co-trimoxazole (80%), Neomycin (80%), Erythromycin (0%), and Amoxicillin (0%). These findings were more or less similar to the findings of Md. Mazedul Haque *et al.* (2017).
From the antibiotic sensitivity test *Enterobacter spp.*, it was found that 100% of the isolated *Enterobacter spp.* was sensitive to Ciprofloxacin followed by kanamycin (75%), Ampicillin (0%), Cefuroxime Sodium (0%), and Amoxicillin (0%). These findings were more or less similar to the findings of Md. Mazedul Haque *et al.* (2017).
Overall more sensitive antibiotics against the isolated organisms was ciprofloxacin followed by Co-trimoxazole, Gentamycin, neomycin, kanamycin and Vancomycin but the isolated organisms were found to be resistant against Amoxicillin, Erethromycin, Ampicillin and Cefuroxime sodium. The variation in the sensitivity of commonly used antibiotic could be a result of extensive and indiscriminate use of these antibiotics, maximum sensitivity to ciprofloxacin, Co-trimoxazole and Gentamycin might probably be due it is rarely used.
There for effective treatment of infection caused by consumption of contaminated betel leaf medicinal formulation should preferably contain antibiotics that have good spectrum of inhibition against the isolated bacteria in this context. It is interesting to note ciprofloxacin, Co-trimoxazole Gentamycin, Vancomycin, Neomycin and kanamycin should be the antibiotic of choice. In deed ciprofloxacin, Co-trimoxazole and Gentamycin could cover most of the infection by prevalent bacteria. Therefore, these antibiotics appear to be promising for the treatment of the infection caused by consumption of infected betel leafs in Bangladesh.

IV. Figures and Tables

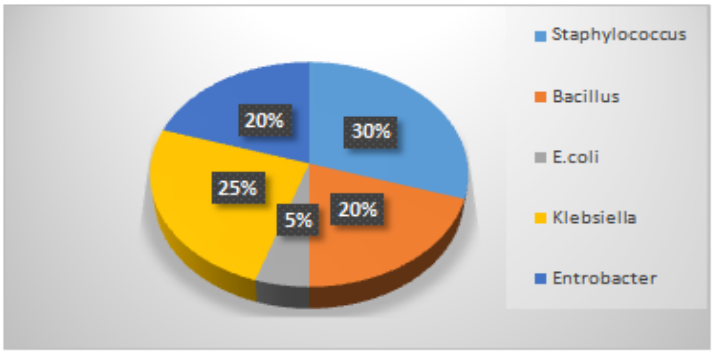


Figure1. Prevalence of bacterial isolates in betel leafs

Yellow colony of*Staphylococcus spp.* on Eosin Manitol salt agar

Hemolytic reaction for *Staphylococcus spp.*On Blood agar media

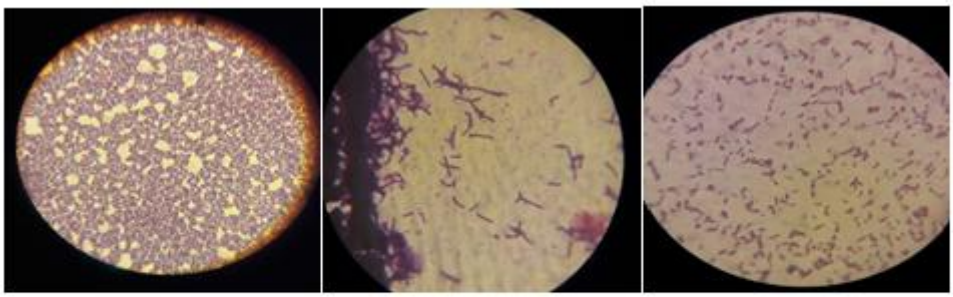
Hemolytic reaction for *Bacillus sp.*On Blood agar media

Metallic sheen colonies of *Escherichia Coli*on Eosin Methylene Blue (EMB) Agar

Pink mucose large coloniesof*klebsiella spp.*on Eosin Methylene Blue (EMB)

Blue colonies of *Entrobacter spp.*on Eosin Methylene Blue (EMB) Agar media

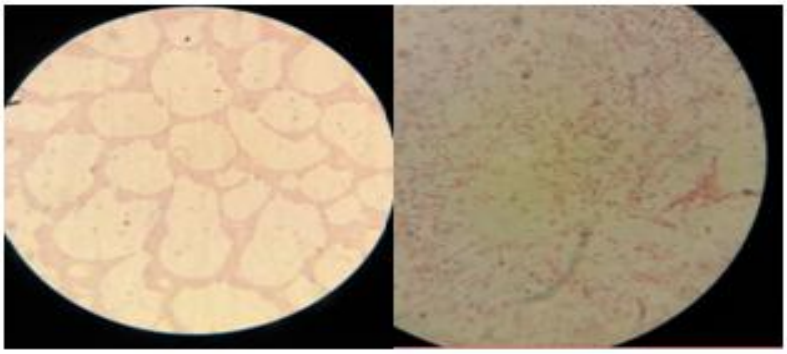
Figure2: Cultural characteristics of bacteria isolated from betel leafs



Violet colored, coccishaped *Staphylococcus spp*arranged in grape likecluster

Violet colored, Rod-shaped *Bacillus spp.*

Pink colored, Rod-shaped *Eshcherichia coli*



Showing pink colored, Rod-shaped *Klebsiella spp* Pink colored, Rod-shaped cluster *Entrobacter spp*

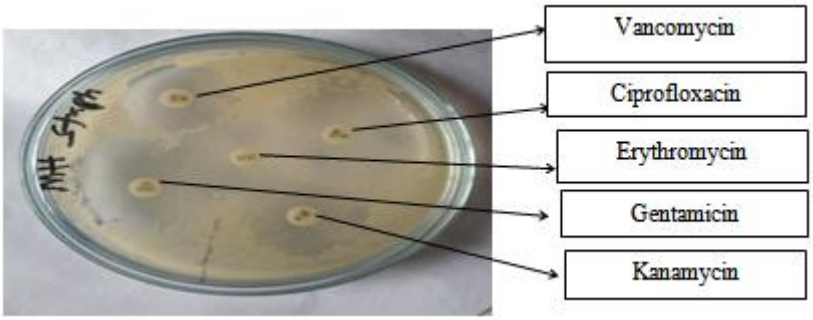


Figure 5.Antibiotic sensitivity test result of *Bacillus spp*. on Mueller Hinton agar

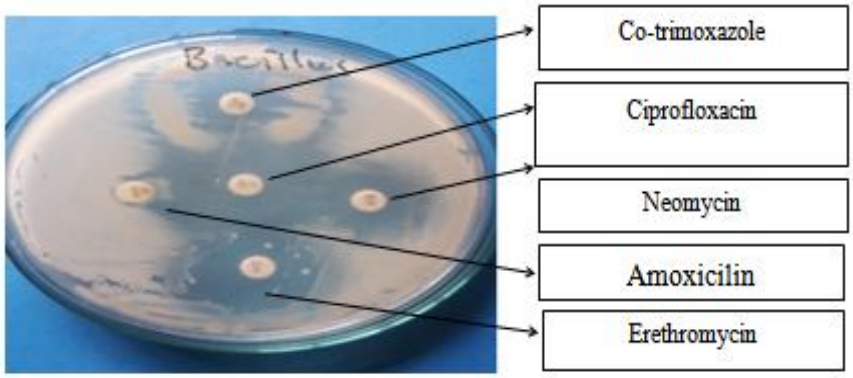


Figure 6. Antibiotic sensitivity test result of *Klebsiella spp.* on Mueller Hinton agar

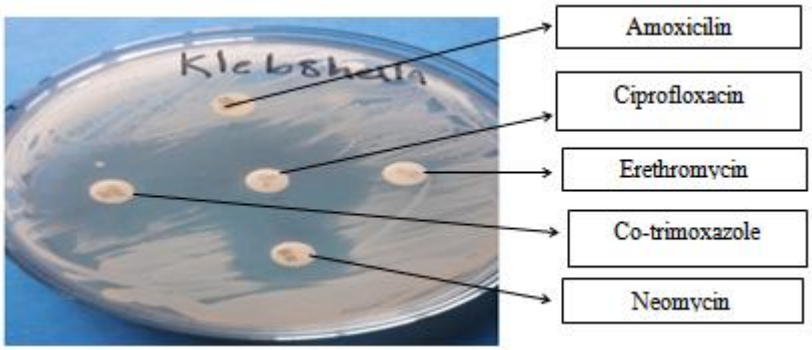


Figure 7.Antibiotic sensitivity test result of *E. coli* on Mueller Hinton agar

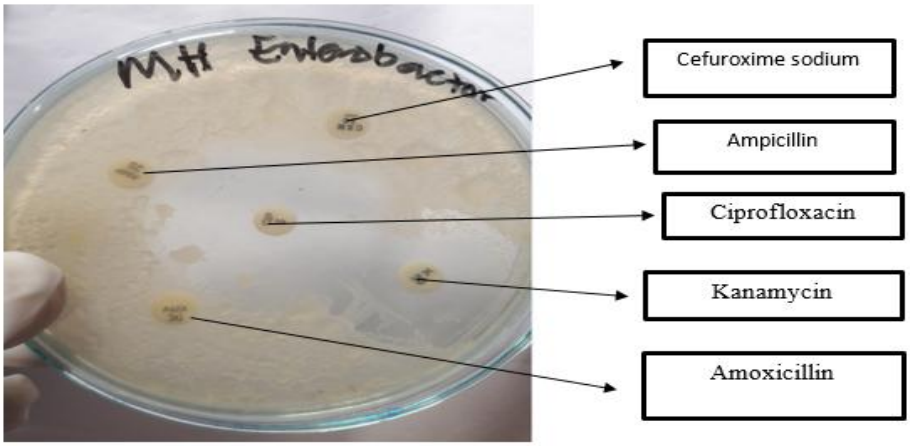


Figure 8. Antibiotic sensitivity test result of *Enterobacter spp.*on Mueller Hinton agar

Table 1**.**Antimicrobial agents with their disc concentration

S1No	Name of the antibiotics	Disc concentration (µg/disc)
1	Ciprofloxacin (CIP)	5 µg/disc
2	Co-trimoxazole (COT)	25 µg/disc
3	Kanamycin (K)	30 µg/disc
4	Neomycin (N)	30 µg/disc
5	Gentamycin (GEN)	10 µg/disc
6	Erythromycin (E)	15 µg/disc
7	Amoxicillin (AMX)	30 µg/disc
8	Vancomycin (VA)	30 µg/disc
9	Cefuroxime sodium (CMX)	30µg/disc
10	Ampicillin (AMP)	10µg/disc

Note: Sl. No. = Serial Number, jig = Microgram,

Table 2.Cultural, Morphological and Biochemical Properties of Isolated *staphylococcus spp.*

Culture characteristics			Biochemical Characteristics		Staining Characteristics
Nutrient Agar	Mannitol Salt Agar	Blood agar	Test	Result	Staining properties
Small Yellow colonies	Yellow colonies	B-type of Hemolytic Were produced	Catalase Indole MIU MR VP TSI Citrate utilization test	+ _ + + + + _	Gram positive cocci arranged in grape like cluster

Table 3.Cultural, Morphological and Biochemical Properties of Isolated *Bacillus ssp.*

Culture characteristics			Biochemical Characteristics		Staining and morphological	India ink staining
Nutrient Agar	Manitol salt agar	Blood agar	Test	Result	Staining properties	Staining properties
Thick, grayish white or, cream colored colonies	Grayish-white colored	B-type of Hemolytic were production	Catalase Indole MIU MR VP TSI Citrate utilization	+ _ _ _ _ + _	Gram-positive, rod-shaped organism	Capsulated

Table4.Cultural, Morphological and Biochemical Properties of Isolated *Escherichia coli.*

Culture characteristics		Biochemical Characteristics		Staining and morphological
MacConkey Agar	EMB Agar	Test	Result	Staining properties
Pink colored colonies	Metallic-sheen color colonies	Indole MIU MR VP TSI Citrate utilization	+ _ + _ _ _	gram-negative, pink-colored, small rod shaped organism arranged in single pairs or short chain

Table 5.Cultural, Morphological and Biochemical Properties of Isolated *klebsiella spp.*

Culture characteristics		Biochemical Characteristics		Staining and morphological
MacConkey Agar	EMB Agar	Test	Result	Staining properties
Pink colored colonies	Smooth pink characteristic mucoid colonies	Indole MIU MR VP TSI SC	_ + _ _ + +	gram-negative, colored,rod shaped organism

Table 6.Cultural, morphological and biochemical properties of isolated *Enterobacter spp.*

Culture characteristics		Biochemical Characteristics		Staining and morphological
MC Agar	EMB Agar	Test	Result	Staining properties
red dry colored colonies	Large mucoid purple colonies	Indole MIU MR VP TSI SC	_ _ _ + + +	gram-negative, colored,rod shaped organism

Legends **(**For Table2,3,4,5,6)**:** MC= MacConkey, EMB=Eosin Methyl Blue, MR=Methyl-red test, VP=Voges-prokauer test, TSI=Triple Sugar Iron, MIU=motility Indole urease test, SC=Simon citrate, + = positive, - = negative.

Table 7.Antibiotic sensitivity pattern of *Staphylococcus spp.*

Antibiotic agent	Disc. concentration (µg/disc)	No. of isolates		Percentages (%)	
		Sensitive	Resistance	Sensitive	Resistance
Kanamycin (K)	30 µg	1	5	16.66	83.33

Erythromycin (E)	5 µg	2	4	33.33	66.66
Vancomycin (VA)	30 µg	4	2	66.66	33.33
Ciprofloxacin (CIP)	30 µg	5	1	83.33	16.66
Gentamycin (GEN)	25 µg	6	0	100	0

Table 8.Antibiotic sensitivity pattern of *Bacillus spp.*

Antibiotic agent	Disc. Concentration (µg/disc)	No. of isolates		Percentages (%)	
		Sensitive	Resistance	Sensitive	Resistance
Amoxicillin (AMX)	10 µg	1	4	25	100
Co-trimoxazole (COT)	15 µg	2	2	50	50
Neomycin (N)	30 µg	3	1	75	25
Ciprofloxacin(CIP)	30 µg	3	1	75	25
Erythromycin (E)	5 µg	4	0	100	0

Table 9.Antibiotic sensitivity pattern of *E.coli spp.*

Antibiotic agent	Disc. Concentration (µg/disc)	No. of isolates		Percentages (%)	
		Sensitive	Resistance	Sensitive	Resistance
Amoxicillin (AMX)	10 µg	0	1	0	100
Erythromycin (E)	5 µg	0	1	0	100
Neomycin (N)	30 µg	1	0	100	0
Co-trimoxazole (COT)	15µg	1	0	100	0
Ciprofloxacin (CIP)	30µg	1	0	100	0

Table 10.Antibiotic sensitivity pattern of *Klebsiella spp.*

Antibiotic agent	Disc. Concentration (µg/disc)	No. of isolates		Percentages (%)	
		Sensitive	Resistance	Sensitive	Resistance
Amoxicillin (AMX)	10 µg	0	5	0	100
Erythromycin (E)	5 µg	0	5	0	100
Neomycin (N)	30 µg	4	1	80	20
Co-trimoxazole (COT)	15 µg	4	1	80	20
Ciprofloxacin(CIP)	30 µg	5	0	100	0

Table 11.Antibiotic sensitivity pattern of *Enterobacter spp.*

Antibiotic agent	Disc. Concentration (µg/disc)	No. of isolates		Percentages (%)	
		Sensitive	Resistance	Sensitive	Resistance
Amoxicillin (AMX)	10 µg	0	4	0	100
Cefuroxime sodium (CXM)	30µg	0	4	0	100
Ampicillin	10 µg	0	4	0	100
Kanamycin	30µg	3	1	75	25
Ciprofloxacin(CIP)	30 µg	4	0	100	0

V. Conclusion

The recent study was conducted for isolation and identification, determination of biochemical properties and antibiotic sensitivity pattern of the bacteria isolated from infected betel leaf samples. A total of 20 betel leaf samples were collected from SadarUpozila Dinajpur district in Bangladesh for this study.

A series of test were conducted for isolation, identification and frequency distribution of different bacteria of betel leaf. A total of 20 bacterial isolates belong to five genera (*staphylococcus spp., Bacillus, spp., Escherichia coli, klebsiella spp and Enterobacter spp*.) were identified. The prevalence of *staphylococcus spp. was* 30%, *Bacillus spp.* was 20%, *Escherichia coli* was 5%, *klebsiella spp*. was 25% and *Enterobacter spp*. was 20%. The result of antibiotic sensitivity tests revealed that *staphylococcus spp* were sensitive to Gentamicin, ciprofloxacin, Vancomycin, Erythromycin, and Kanamycin in various degrees. *Bacillus spp* were sensitive to Erythromycin, Ciprofloxacin, Neomycin, Co-trimoxazole, in various degrees. *E. coli* was sensitive to Ciprofloxacin, Co-trimoxazole, Neomycin in various degrees. *Klebsiella spp* were sensitive to Ciprofloxacin, Co-trimoxazole, Neomycin in various degrees. *Enterobacter spp* was sensitive to Ciprofloxacin, Co-trimoxazole, Neomycin in various degrees. Overall sensitivity revealed that ciprofloxacin, Co-trimoxazole and Gentamycin were most efficacious drugs. Therefore; it may be recommended that ciprofloxacin, Co-trimoxazole and Gentamycin in optimum doses to treat most case of infection caused by consumption of betel leaf at SadarUpazila in Dinajpur district in Bangladesh.From the result of the present study, it may be concluded that.

1. *Staphylococcus spp, Bacillus spp, E. colispp, klebsiella spp, Enterobacter spp* were the major etiological agent isolated from betel leafs.
2. Antibiogram result indicated the ciprofloxacin, Co-trimoxazole and Gentamycin, in optimum doses would be the drug of choice to treat the most cases of human infection caused by consumption of infected betel leafs.

3. Antibiotic sensitivity test revealed that Ampicillin, Cefuroxime Sodium, Amoxicillin and Erythromycin would not be recommended because isolated bacteria were resistant to these drugs.

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