



Microbial characterization of spoiled food and the screening for the potential antibiotics that inhibit the identified food pathogens

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Abstract:

In the current era of fast and corporate life style one of the major problems faced by the individuals is the health hazards caused by spoiled or improperly maintained food. Currently there is an extensive increase in the usage of packaged and stored foods that would always be a hub for all the diseases caused by various food borne pathogens. In view of these problems the current work aims to characterize the microbial pathogens in selected spoiled food samples followed by their identification. It involves the isolation of bacteria from the selected spoiled food samples, Preparation of their pure cultures followed by their identification based on Biochemical tests as per Bergey's Manual. Once the bacterial pathogens are identified, the study was extended towards the screening of the antibiotics that are capable of inhibiting the growth of these pathogens. The work concludes by proposing the specific antibiotics that can be used towards the infections or diseases caused due to the consumption of spoiled food contaminated with the specific organism under study. Once the results are validated using the clinical study they can be applicable directly for treating the abnormalities caused by food borne pathogens. The work can be further extended toward the identification of fungal pathogens.

Key words: *Bergey's Manual, Contamination, Pathogens, Antibiotics.*

Introduction:

Spoilage is a complex process and it involves physical, chemical, biochemical and biological changes [1]. Microbial contamination of ready-to-eat foods and beverages sold by street vendors has become a global health problem [2]. An analysis of the street vended food stuffs revealed the presence of the bacteria *Staphylococcus faecalis*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus Species*,

Klebsiella species and *Pseudomonas species* as the contaminating pathogens [3]. It is a metabolic process that causes Foods to be undesirable or unacceptable for human consumption due to changes in sensory characteristics [4]. Most of the spoilage is due to the microbial contamination of the food either by bacteria or by fungi. Bacteria are the first contaminants in the food when compared to fungi. Microbial pathogens in food cause an estimated 6.5-33 million cases of human illness and up to 9,000 deaths in the United States each year [5]. Foodborne pathogens are responsible for millions of cases of infectious gastrointestinal diseases each year, costing billions of dollars in medical care and lost Productivity [6]. Factors that affect food spoilage are pH, moisture content, water activity, oxidation-reduction potential, physical structure of the food, available nutrients, presence of antimicrobial agents, temperature, relative humidity, carbon dioxide or oxygen and type and numbers of microorganisms in the food. Routes of microbial contamination of food are air, dust, soil, water, plants, animals, animal feed, food handlers, and food utensils, cross contamination, unsafe temperature and pests [7]. The microbes will not only cause the food to be undesirable for consumption but also causes many dreadful diseases when consumed. The diseases caused due to the food born pathogens mostly alter the gastrointestinal tract and throat condition. They often cause diarrhoea, Nausea, Vomiting, dehydration etc. The cases reported with these symptoms and causes are enormously increasing with the advancement in the use of packaged food products. Most of these bacteria can be inhibited from contaminating the food by maintaining high acidity and low water activity level.

Materials and Methods:

Isolation of bacteria from contaminated food samples: Eight spoiled food samples infected with bacteria were collected (Dal, Plain Rice, Tomato

Rice, Curd, Noodles, Brinjal, Beans and Upma) from different regions of Hyderabad and brought to laboratory the laboratory. A piece of each sample was taken with the help of a sterile forceps and used for inoculation onto sterilized petri dishes containing sterile nutrient agar medium. The plates were incubated at 37°C for 24 hours in an inverted position. This step produces the master plate containing various types of bacteria in the spoiled food samples

Preparation of pure culture: After 24 hours the incubated plates were observed for the colony morphology of the colonies. Different colonies were selected based on the morphology and were used as an inoculum for pure culture preparation. The pure plates were further subjected to sub culturing to retain the purest culture of the colony. The sub culturing can be performed by taking a loop full of culture from each master plate and making a quadrant streak on the pre solidified media. The plates were incubated at 37°C for 24 hours. The colony morphology was studied from pure cultures for each sample. The culture from these pure cultures can be used for further analysis and characterization.

Gram Staining: To identify the eight organisms selected in the previous step the respective smears were prepared by using a loop and a glass slide. The smear prepared is air dried and used for Grams staining. Once the gram staining protocol is done than the slides are subjected for heat fixing by passing the slide against a flame source so as to fix the microbes on to the slide and dry the culture. Observe the slides under 45x and 100x oil immersion objective to identify the morphology and gram nature. The result for all the eight slides was recorded. Gram-positive cells appear purple and Gram-negative cells pink. The morphology can be noted as either cocci or Bacilli along with the other characteristics. The identified bacteria are subjected to Biochemical tests based on Bergey's Manual.

Biochemical tests:

To identify the organisms obtained in the previous tests, the biochemical characters must be screened. The tests performed are as under.

Catalase Test: This test aims to detect the production of an enzyme Catalase by the test organism. The enzyme will hydrolyze the hydrogen peroxide to water and oxygen. The test is performed by placing all the slides in a laminar flow to which one drop of hydrogen peroxide is added. To the above drop one loop full of the culture is added. If the addition causes immediate brisk effervescence, it indicates the presence of catalase and no reaction would be an indication of absence of catalase.

Starch Hydrolysis Test: This tests aims to identify the capability of the organisms to digest the starch by producing the enzyme amylase. Soluble starch is dissolved in nutrient agar, sterilised in an autoclave at 121°C at 10lbs pressure, poured into petri plates and allowed to solidify. Bacterial inoculum from pure cultures are inoculated onto the plates. Plates are incubated at 37°C for 24–48 hrs. To test the hydrolysis of starch, each plate is flooded with iodine. Iodine reacts with starch to give a blue colour complex. Digestion of the starch by amylases of bacteria will produce a zone of hydrolysis. All the cultures were tested for the amylase enzyme.

Mannitol Fermentation Test: This is to test the ability of the bacteria to digest Mannitol and ferment it into simple sugars with the release of gas. The media is incorporated with mannitol sugar and Phenol red is used as a pH indicator for the detection of acid formation. Durham's tubes as used to detect the release of gas. The medium along with the Durham's tubes is sterilized and then cooled to room temperature. The test organisms are inoculated into separate tubing's under sterile conditions. The tubes are incubated and observed for the colour change and gas production. The change in the colour from red to yellow indicates the formation of acid and bubble formation in the Durham's tubes indicates the release of gas.

Voges Proskauer Test: Sterile MR-VP broth tubes were prepared and sterilized. The tubes were inoculated with the inoculum from the pure culture plates. The tubes were further incubated for 24 to 48 hours at 37°C. To the tubes 10 drops of Barrett's A reagent and 10 drops of Barrett's B reagent was

added. Red colour formation within 15 to 20 min indicates a positive test.

Swollen Cell Test: Sterile swollen cell media broth tubes were prepared. Inoculum was taken from the pure culture plate and was transferred to the broth tubes. The tubes were then incubated for 24 to 48 hours at 37°C. After incubation the colonies were picked and subjected for simple staining with saffronin. The slides were air dried and the smear was observed under 100x oil immersion objective. The appearance of the cells larger than the normal size indicates the positive result for swollen cell test.

Simmons' Citrate Test: Sterile citrate agar slants were prepared onto which the test samples were streaked on the surface of the slants. The slants were incubated at 37°C for 24 hours. The change in the colour of the medium from green to blue indicates the positive test due to the presence of citric acid. No change in the colour indicates the absence of citric acid.

Screening of the inhibitory activities of commercial antibiotics against the identified bacterial Pathogens: Muller Hinton Agar was prepared and sterilized. The media was poured into 8 petri plates for testing the 8 organisms under study. The test organisms were inoculated onto the plate by spread plate method. Onto the inoculated plates wells were punched so as to add the test antibiotic. Add 100 Micro liters of the antibiotic into the wells. The plates were incubated for 24 hrs at 37°C. The plates were observed for the formation of zones of inhibition. Greater the zone of inhibition greater is the activity of the antibiotics against the test pathogen.

Results and Discussion:

Observation of colony morphology of the colonies on the master plate: On the master plate there were several colonies with similar out look and only few colonies must be selected that vary from one another in their colony morphology so as to prepare the pure cultures. The morphologies observed were as under:

Sample 1, Sample 2 and Sample 7: Regular Margin, Convex, Rough surface, White coloured

colonies. **Sample 3:** Irregular Margin, Concave, Smooth Surface, Transparent colonies.

Sample 4 and Sample 5: Regular Margin, Concave, Smooth surface, White coloured colonies.

Sample 6: Regular Margin, Concave, Smooth surface, Transparent colonies

Sample 8: Irregular Margin, Concave, Smooth surface, white coloured colonies.

Gram's staining: The test results for grams staining to all the 8 cultures are as below: Sample 1, 3, 6, 7, 8 is Gram positive sporulating bacilli. Sample 2 is a gram positive cocci and sample 4 and 5 were gram positive non- sporulating cocco-bacilli.

1: Results for Biochemical tests for the 8 test organisms as per Bergey's Manual

Test	S1	S2	S3	S4	S5	S6	S7	S8
Catalase test	+	+	+	+	+	+	+	+
Starch Test	+	+	+	+	+	+	+	+
Mannitol fermentation test	-	-	NA	NA	NA	-	-	-
Voges-Proskauer(VP) TEST	+	NA	-	NA	NA	+	+	+
Swollen cell test	NA	NA	-	NA	NA	NA	NA	NA
Simmons' Citrate Test	-	NA	-	NA	NA	-	-	-

NOTE: S1- Sample 1, S2- Sample 2, S3- Sample 3, S4- Sample 4, S5-Sample 5, S6-Sample 6, S7- Sample 7, S8-Sample 8.

The **table 1** shows the complete biochemical properties of all the test organisms based on which they are characterized and identified. From the table the organisms were confirmed to be *Bacillus alvei*, *Staphylococcus species*, *Bacillus badius*, and *Corynebacterium kutscheri*.

Antibiotic tests for the pathogens: To test the activity of various commercially available antibiotics on the test bacterial pathogens.

Zone of inhibition test was performed with the antibiotics, Tetracycline (Tet), Azithromycin (Az), Clotrimazole (Cl) and Ampicillin (Amp). The antibiotic were diluted and were used in the volume of 100micro liter per well. The organisms were spread and the antibiotics were added onto the screen plates. The plates were observed after the incubation period. The results were documented as below:

Table 2: showing the inhibitory effect of different antibiotics on the test organism

Sample number	Name of the microorganism	Tet (mm)	Az (mm)	Cl (mm)	Amp (mm)
1	<i>Bacillus alvei</i>	14	13	12	15
2	<i>Staphylococcus spp.</i>	16	18	15	12
3	<i>Bacillus badius</i>	19	17	15	14
4	<i>Corynebacterium kutscheri</i>	19	NZ	19	NZ
5	<i>Corynebacterium kutscheri</i>	16	NZ	16	NZ
6	<i>Bacillus alvei</i>	19	13	16	14
7	<i>Bacillus alvei</i>	19	16	14	14
8	<i>Bacillus alvei</i>	16	15	15	15

NZ- no zone of inhibition.

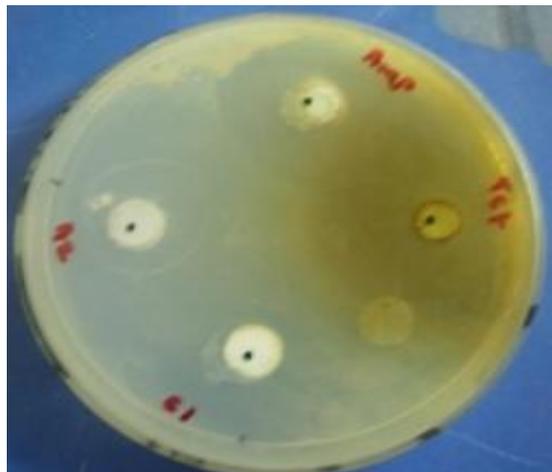
The above Table 2 shows the zones of inhibition obtained for the different antibiotics against the test microbial pathogens. It is clear from the above table that all the antibiotics tested had good inhibitory effect on the selected food pathogens except in few cases. This result proposes on the use of these antibiotics in the treatment of minor ailments due to food borne pathogens. The zones of inhibition obtained with the antibiotics are depicted in the figure 1 and 2.

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Figure 1 showing the zones of inhibition obtained for the organism *Corynebacterium Kutscheri* against the four selected antibiotics.



Figure 2 showing the zones of inhibition as obtained for the test organism *Bacillus alvei* against the four selected antibiotics.



Conclusion

From the current work it is identified that the most common organisms inhabiting the spoiled foods are *Bacillus alvei*, *Corynebacterium kutscheri*, *Bacillus Badius* and *staphylococcus species*.

Once the bacterial pathogens were identified the work was further extended to screen the antibiotic sensitivity of these test bacteria against the four selected antibiotics, Tetracycline, Azithromycin, Clotrimazole and Ampicillin. The results from the sensitivity study showed that all the selected antibiotics had a good inhibitory activity against the test pathogens except in case of *Corynebacterium kutscheri* to the antibiotics Ampicillin and Azithromycin.

From the results furnished above it can be concluded that these selected antibiotics can be used in the treatment of the minor ailments caused due to the food borne pathogens. Further the use of these antibiotics within the limited concentration in the preservative foods can be emphasized; however this can be validated only with the support of the clinical data.

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