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Identification and Characterization of Food Borne Microorganisms, With A Complete Analysis for Its Antidrug Potential Against Major Antibiotics And *"Allium Sativum"* Aneous Decoction

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Abstract

There has been growing awareness of the major public health impact of zoonotic food borne pathogens from foods of animal origin. Several epidemiological reports have implicated foods of animal origin as the major vehicles associated with illnesses caused by food-borne pathogens. Contaminated raw or undercooked poultry and red meat remains the most important source of human infection with the most commonly reported food borne pathogens. Food borne pathogens is the main etiological agents of illness and death in developing countries. Salmonella, *Escherichia coli*, and *Staphylococcus aureus* are the main predominant species in most food poisoning cases. Antimicrobial agents are extensively used in the poultry industry for disease prevention or as growth promoters. Wide spread of antibiotic-resistant food borne pathogens threaten the successful treatment of infectious diseases. The detection and enumeration of microorganisms in food are an essential part of any quality control or food safety plan. Traditional methods of detecting food- borne pathogenic bacteria are often time-consuming because of the need for growth in culture media, followed by isolation, biochemical and/or serological identification, and in some cases, subspecific characterization. Advances in technology have made detection and identification faster, more sensitive, more specific, and more convenient than traditional assays.

Keywords

Food borne, *Escherichia coli*, Staphylococcus *aureus* and Microorganisms

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INTRODUCTION:

There has been growing awareness of the major public health impact of zoonotic food borne pathogens from foods of animal origin. Several epidemiological reports have implicated foods of animal origin as the major vehicles associated with illnesses caused by food-borne pathogens. Contaminated raw or undercooked poultry and red meat remains the most important source of human infection with the most commonly reported food borne pathogens. Food borne pathogens is the main etiological agents of illness and death in developing countries. Salmonella, Escherichia coli, and *Staphylococcus aureus* are the main predominant species in most food poisoning cases. Antimicrobial agents are extensively used in the poultry industry for disease prevention or as growth promoters. Wide spread of antibiotic-resistant food borne pathogens threaten the successful treatment of infectious diseases.

The detection and enumeration of pathogens in food and on surfaces that come into contact with food are an important component of any integrated program to ensure the safety of foods throughout the food supply chain. Microbiological analysis is also an essential tool for carrying out tests in accordance with the microbiological criteria established for each food type, as well as being essential for evaluating the actions of different management strategies based on the Hazard Analysis and Critical Control Points (HACCP) system[7&5.] Major food pathogens are *salmonella, E. coli, Bacilli, S. aureus*. The level of pathogenicity is depending on the range of contamination. Each micro organism has its own characteristic features.

Salmonella is a non-spore-forming rod-shaped, mostly motile Gram-negative bacterium belonging to the family Enterobacteriaceae, in which approximately 2,200 serotypes are recognized. Foods commonly associated with the disease include raw meats, poultry, eggs, and milk and dairy products. Milk-borne *salmonellosis* is common in parts of the world where milk is neither boiled nor pasteurized. It occurs, but much less frequently, in developed countries where the main products implicated are pasteurized milk, powdered milk and certain cheeses. Several groups have reported that Salmonella has formed biofilm on various types of surfaces used in the food processing industry. These studies have shown that Salmonella spp. can form biofilms on food contact surfaces and that the cells in biofilms are much more resistant to sanitizers compared with planktonic cells.

The evolution and spread of antibiotic resistance as well as the evolution of new strains of disease-causing agents is of great concern to the global health community. Our ability to effectively treat disease is dependent on the development of new pharmaceutical, and one potential source of novel drugs is traditional medicine. This study explores the antibacterial properties of plants used in Haudenosaunee traditional medicine. We tested the hypothesis that extracts form Haudenosaunee medicinal plants used to treat symptoms often caused by bacterial infection would show antibacterial properties in laboratory assays and that these extracts would be more effective against moderately virulent bacteria than less virulent bacteria. Frank M Frey Ryan Meyers., (2010).

There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases. Another big concern is the development of resistance to the antibiotics in current clinical use. In recent years, drug resistance to human pathogenic to bacteria has been commonly reported from all over the world. In the present scenario of emergence of multiple drug resistance to human pathogenic organisms, this has necessitated a search for new antimicrobial substances from other sources including plants. Higher plants produce hundreds to thousands of diverse chemical compounds with different biological activities. The antimicrobial compounds produced by plants are active against plant and human pathogenic microorganisms. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug-resistant microbial pathogens. In the present study, we have selected some medicinal plants to be screened against multi-drug resistant bacteria including Staphylococcus aureus, pseudomonas aeruginosa, Escherichia coli, and various multiple drug resistant fungi (Singh, *et al*., 2010).

In rural and backward area of West Bengal, India several plants are commonly used as herbal medicine for the treatment of infectious diseases. Four such plants commonly used by the people of the area were

screened for potential antibacterial activity. Antibacterial activity of both aqueous and methanol extracts of the plant's parts were used for screening. The plants screened were sodium guajava, Andrographis paniculata, Terminalia arjuna and Adharoda vasica. Antibacterial activity was tested against six strains of both Gram positive and negative bacteria. The susceptibility of the microorganisms to the extracts of the extracts of these plants was compared with each other and with selected antibiotics. The result showed that, the methanol extracts of selected medicinal extracts of selected medicinal plants exhibited high activity against the tested organisms rested organisms rested organisms rather than aqueous extract of those plants. So, the minimum inhibitory concentration (MIC) of the methanol extract of selected plants was studied. Extract form Terminalia Arjuna showed higher antimicrobial activity the extract of psidium guajava.

The development of new antimicrobial agents against multidrug resistant pathogens for the treatment of skin infections is of increasing interest. Therefore, the aqueous and ethanolic extracts from different parts of five medicinal plants used locally in folk medicine were evaluated for antimicrobial activity against the most frequent skin pathogens. It was found that most plant extracts studied had antibacterial and antifungal activities. The antibacterial activities with the best minimum inhibitory concentration values were significantly produced by the aqueous extracts of *Eminium spiculatum* stems and *Lupinus varius,* seeds against Escherichia coli, and *Methicillin*-resistant extracts of *mandragora autumnalis,* fruits against Escherichia coli, and *Methicillin*-resistant *Staphyloccus aureus,* whereas, the highest significant antifungal activity with the best value was produced by aqueous extracts of L.varius seeds against Candida albicans. However, leaf extracts of the tested plants were appeared to produce the least antimicrobial activity. It was concluded that the antimicrobial activity is associated with the used part of plant in addition to the type of solvent used for extraction. The antimicrobial effects of some plant extracts, in particular aqueous seed extracts of L.varius and ethanolic fruit extracts of M.autum nalis, may be for the topical treatment of skin infections.

Neurohormonal mechanisms, pathogens, malnutrition, chronic diseases and drugs can alter gastrointestinal physiology resulting in changes in either secretion or absorption of fluid by the intestinal epithelium. Altered motility contributes in a general way to this process, as the extent of absorption by and large parallels transit time. Prokinetic agent's bowel syndrome collagen vascular disease is some of the pathophysiological conditions that may alter intestinal motility and transit time. Antimotility compound such as diphenoxylate lopera mid opium alkaloids anticholinergics ete. Have been tried against diarrheal disorders but often with side effects after prolonged use.

Clinical microbiologists have two reasons to interest in the topic of antimicrobial plant extracts. First if is very likely that these phytochemicals will find their way into the arsenal of antimicrobial drugs prescribed by physicians[3]. Several are already being tested in humans. It is reported that on average two or three antibiotics derived from microorganisms are launched each year. After a downturn in that pace in recent decades the pace is again quickening as scientists realize that the effective life span of any antibiotic is limited. Worldwide spending on finding new anti-infective agents (including vaccines) is expected to increase 60% from the spending levels in 1993. New sources, especially plant sources are also being investigated. Second the public is becoming increasingly aware of problems with the over prescription and misuse of traditional antibiotics. In addition, many people are interested in having more autonomy over their medical care. A multitude of plant compounds (often of unreliable purity) is readily available over–the–counter form herbal suppliers and natural food stores and self-medication with these substances is commonplace. The use of plant extracts as well as other alternative forms of medical treatments is enjoying great popularity surveyed in the United States used at least one unconventional therapy during the previous year. It was reported that in 1996 sales of botanical medicines increased 37% over 1995. It is speculated that the American public may be reacting to over prescription of sometimes toxic drugs just as their predecessors of the 19th century reacted to the overuse of bleeding purging and calomel.

Moerman suggested that North Americas history of plant medicinal use follows tow strands-their use by indigenous cultures[6] (Native Americans), dating from

prehistory and an alternative movement among Americans of European origin beginning in the 19th century. Native American use of plant medicinal has been reviewed extensively in a series of articles by Moerman he reported that while 1,625 species of plants have been used by various Native American groups as food 2,564 have found use as drugs. According to his calculations this lives approximately 18, 00 species of plants which were used for neither food nor drugs. Speculations as to how and why a selected number of plant species into use for either food or drugs is fascinating but outside the scope of this review.

Described an anthraquinone from *Cassiaitalica*, a Pakistani tree, which was bacteriostatic for *Bacillus anthracis, Corynebacterium pseudodiphthericum,* and *pseudomonas aeruginosa* and *pseudomonas aeruginosa* and bactericidal for *pseudomonas pseudomalliae.* Hypericin, an anthraquinone form St. John's wort (*Hypericum perforratum*), has received much attention in the popular press lately as an antidepressant, and Duke reported in 1985 that it had general antimicrobial properties.

Eloff J.N. He examined a variety of extractnts[4] for their ability to solubilize antimicrobials form plants as well as other as other factors such as their relative ranking as biohazards and the ease of removal of solvent form the fraction. The focus of the study was to provide a more standardized extraction method for the wide variety of researchers working in diverse settings. Although it is not one of the more frequently used extractants in studies published to date acetone received the highest overall rating. In fact, in a review of 48 articles describing the screening of plant extracts for antimicrobial properties in the most recent years of the journal of which are reported in the recent literature with the highest frequency Eloff ranked them in the order methylene dichloride methanol ethanol and water.

The row indicating the number of inhibitors extracted with each solvent points to tow implications first that most active components are not water soluble supporting the data reported second that the most commonly used solvents (ethanol and methanol both used as initial extractants in approximately 35% of the studies appearing in the recent literature) may not demonstrate the greatest sensitivity in yielding antimicrobial chemicals on an initial screening. This disparity should be examined as the search for new antimicrobials intensifies.

MATERIALS AND METHOD:

Collection of test samples

Various ice cream and fish samples were collected from the local area as per the traditional collection procedure of cold food samples.

Chemicals Used

All chemicals used for this study were high quality analytical grade reagents. The solvent search as ethanol water and hexane were purchased from S.D. fine chemicals pvt. Ltd., Sigma chemical, Merck chemicals supplies and Hi media.

Inoculum preparation

Ten milliliters of thawed samples were dispersed in 90ml of sterile distilled water to obtained 10^{-1} dilution. Further dilution was made by transferring 1ml into 9ml distilled water until 10^{7} dilutions was obtained. Aliquot portion (0.1ml) of 10^{7} and 10^{6} dilution was inoculated surface dried nutrient and MacConkey agar respectively. The same quantity (0.1ml) was transferred from 10^{5} and 10^{4} into LB media. Inocula were spread evenly and plates incubated at recommended temperature and time [1&2].

MICROBIOLOGICAL ANALYSIS OF SAMPLES

Preparation of IC for the bacteriological analysis

The ice cream samples were allowed to melt at 5^{0}c under aseptic condition, after which they were used for the analysis. The total colony count was done by pour plate method using nutrient agar. The ice water samples were serially diluted using sterile distilled water, and 0.2 ml of appropriate dilution was used to inoculate the plate in duplicate. The plates were incubated at 37^{0}c for 24 h, after which the total colony count was determined. Distinct colonies based on colonial morphology were purified to obtain pure cultures that were subjected to routine primary and biochemical tests. The isolates were identified according to the scheme of the three-tube procedure using lactose broth was used to detect the coliform and determine the most probable number (MPN) of coli form bacilli using McCrady table. A 0.1 ml, 1 ml, and 10 ml of each sample were used to inoculate the lactose broth in five replicates. Tubes were incubated at 37 °C for 48 h and the MPN was determined in accordance with standard method (APHA, 1985). For

the detection of fecal coliform bacteria, production of acid and gas was taken as positive indication.

Assay for pathogenic load of fish sample.

0.1 ml of each sample was spread onto nutrient agar, membrane fecal coliform (mFC) agar, Sabouraud dextrose agar (SDA) and mannitol salt agar (MSA) for enumerating total viable bacteria (TVB), total fecal coliform (TFC) and Staphylococcus aureus, consecutively. For TVB and staphylococcal assay, plates were incubated at 37o C for 24 hours while for fecal coliforms, plates were incubated at 44.5o C for 24 hours. For the isolation of Escherichia coli and *Klebsiella* spp., 0.1 ml suspension was spread over MacConkey agar and incubated at 37o C for 18- 24 hours. Presence of *E. coli* was further confirmed by the appearance of bluish-black colonies with green metallic sheen on eosin-methylene blue (EMB) agar.

One ml of serially diluted sample was transferred to enrichment media for Salmonella spp., and also to the alkaline peptone water for the enrichment of Vibrio spp., followed by incubation at 37° C for 6 hours. From each of the 10-4 to 10-6 dilutions of the enriched broth, 0.1 ml of suspension was spread onto SS (Salmonella – Shigella) agar plates. After incubation at 37°c for 24h, characteristic colonies were enumerated. One ml of homogenized sample was transferred to 9 ml of selenite cystine broth for the enrichment of Shigella spp. and Salmonella spp., and also to the alkaline peptone water for the enrichment of vibrio spp., followed by incubation at 37°c for 6 hours. From each of the 10^{-4} to 10^{-6} dilutions of the enriched broth, 0.1 ml of suspension was spread onto Xylose Lysine Deoxycholate (XLD) and Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar plates. After incubation at 37°c for 24h, characteristic colonies were enumerated and subjected for Gram studies and biochemical analysis.

Coliform and Faecal Coliform Count

The Most Probable Number (MPN) method was used to determine total coliforms, faecal coliforms and E. coli for all the three food commodities. The samples were subjected to Lauryl Tryptose broth (L T) (OXOID) following the 3 x 3-tube method (Andrews 1992). The tubes were incubated for 48 hours at 35°C. Aloopful of cultures showing gas production from the tubes was streaked on Eosin Methylene Blue agar (EMBA) (OXOID) to confirm presence of coliforms. The presence of E.coliwas an indication of faecal contamination.

Identification of *E. coli*

0.1ml from the pre-incubated samples in peptide water was streaked onto MacConkey agar (CM0007, Oxoid) plates and incubated at 37°C for 24 h. Following incubation, lactose-positive colonies (3-5) were streaked onto Eosin-methylene blue agar plates. Typical *E. colicolonies* on eosin-methylene blue agar (green and shiny or with dark or purple centers) were sub cultured in nutrient agar sland.

IDENTIFICATION PARAMETERS

Staining technique

Gram's staining was discovered in German bacteriologist Dr. Physician Gram. This method used to differentiate 2 important groups of bacteria as gram positive, gram negative. Materials used in gram staining as crystal lioled, gram's iodine alcohol saffranin. Crystal violet used as a primars stain. Grams iodine mordant stain to forms an insoluble complex by binding the primary stain to form CVI complex. Alcohol discoloring agent. saffranin counter stain.

Make a thin bacterial smear on a grease free class slide and mark near the edge of the slide. Allow these near to air dry and fixed with heat. Flood thes smear with crystal riolt and allow it 30 second wash the smear in running tap water. Stain the smear of iodine solution and allow it 40 seconds. decolouring the smear with the help of alcohol. Then finally add counter stain of sffrning for 30 seconds. wash the slide with disal water and allow to air dry observe and er microscope.

BIOCHEMICAL CHARACTERIZATION:

INDOLE TEST

To differentiate the organism *E. coli* and *klebsiella* on the basics of indole production. Tryptophane is an essential amino acid is oxidized by some bacteria by the enzyme trytophanase resulting in the formation of indole, pyruvic acid and ammonia. The indole test is performed by inoculating a bacterium with tryptophane broth, the indole production during the reaction is detected by kovac's reagent dimethylamino benzaldehyde which produces a cherry red colour.

METHYLRED TEST

Glucose is being oxidized by enteric organisms and the end products very based on the metabolism. The methyl red test is employed to detect the ability of organism to oxidize glucose with the production of acids like formic, acetic, lactic and succinic acid. Although all enteric forms ferment glucose with

organic acid, it is used to differentiate particularly *E. coli* and *klebsiella*.

VOGES-PROSKAUER TEST

The voges- proskauer test determines the ability of some microorganisms to produce non-acid or neutral and products such as acetyl methyl carbinol.

Oxidation of acetyl methyl carbinol to a diacethy compound is essential for the detrction of methyl carbinal. The oxidation wills accus under alkaline conditions in the present of the catalyst, a-naphtha and a guanidine group which is present in the peptone of MR-VP medium.

CITRATE UTILIZATION TEST

In the absence of fermentable glucose or lactose some microorganisms are capable of utilizing citrate as the carbon source for this energy. The ability depends on the presence of enzyme citrate in cell. Citrate is acted on by the enzyme citrase which produces oxaloacetic acid and acetate. These products are then enzymatically converted into pyruvic acid and co_2. During this reaction the medium becomes alkaline and the generated co_2 combines with sodium and water to form sodium carbonate which changes bromothymol blue indicator to form deep blue or green colour.

OXIDASE TEST

To determine the oxygen requirement of the bacteria. To demonstrate the presence of oxidase enzyme. To demonstrate metabolic nature of bacteria.

UREASE

The test organism cultured in a medium containing urea with indicator phenol red. It the organism is urease producing, the enzyme will break down the urea (hydrolysis) to give ammonia&co_2.

Urea $\xrightarrow[\text{Hydrolysis}]{\text{urease Ammonia}}$ **$+co_2+H_2o$.**

With the release of ammonia, the medium becomes alkaline as shown by a change in the colour of the indicator red to pink. The medium used here is Christensen's urea agar which contains less buffer. Organisms that are strong urease producing, cause medium to turn red.

GROWTH KINETICS UNDER DIFFERENT TEMPERATURE

To know the growth level difference of identified micro organisms under various temperature condition, 20 ml of LB Broth was taken in different conical flask along with inoculated culture. These flasks were kept in three different temperature settings, such as $^{-}2\ ^{0}C$, $37^{0}C$ and $50^{0}C$. All flasks were subjected for shaking mode during their incubation time. After 14 h incubation, all flasks were considered for the UV-Spectrophotometer analysis. Optical density (OD) values were taken at 600nm.

DETERMINATION OF ANTIMICROBIAL SUSCEPTIBILITY

Following the identification of different colonies, the confirmed isolates were spread on Mueller-Hinton Agar (oxoid) and the antibiotic discs were placed over the plate and incubated at 37°C for 18-24 h according to Clinical and Laboratory Standard Institute (CLSI., 2015). The criterion for the antibiotic chosen was based on their use in both food production and human therapy. The antibiotics used in this study are *Erythromycin, Chloramphenicol* and *Kanamycin* at different concentration level. The clear zone around each antibiotic disc was measured in millimeter. Strains were evaluated as susceptible, intermediate or resistant.

EVALUATION OF HERBAL BASED INHIBITORY ACTION AGAINST PATHOGENS

Selected Plant Species:

Allium sativum (Lehsun) was selected to evaluate their antibacterial activity. It was purchased from the local vegetable market (Mettur, TamilNadu).

Crude Extract:

The crude extract was prepared by method explained by Abubakar. Fresh plant material was being collected and then washed under tape water for 2-3 times for the removal of extra debris, mud, etc. It was then cut into fine pieces and again washed with the distilled water for clearing of material. It was then crushed with pestle and mortar till fine paste was obtained. It was then filtered with the help of Watman filter paper no.1, filtered solution was centrifuged at 10000rpm for 10minutes. Pellet was discarded and supernatant was be used for experimental work.

Spot screening for Phyto chemicals

Phytochemical analysis of different Crude extracts

Extracts were tested for the presence of active principles such as Triterpenoids, Steroids, Glycosides, Saponins, Alkaloids, Flavonoids, and Tannins.

Test for Steroids and Triterpenoids:

Liebermann Burchard test - Crude extract was mixed with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was then added from the sides of the test tube and observed for the formation of a brown ring at the junction of two layers. Green coloration of the upper layer and the formation of deep red color in the lower layer would indicate a

positive test for steroids and triterpenoids respectively.

Test for Glycosides:

Keller Killiani Test – Test solution was treated with few drops of glacial acetic acid and Ferric chloride solution and mixed. Concentrated sulphuric acid was added and observed for the formation of two layers. Lower reddish-brown layer and upper acetic acid layer which turns bluish green would indicate a positive test for glycosides.

Test for Saponins:

Foam Test – Test solution was mixed with water and shaken and observed for the formation of froth, which is stable for 15 minutes for a positive result.

Test for Alkaloids:

Hager's Test – Test solution was treated with few drops of Hager's reagent (saturated picric acid solution). Formation of yellow precipitate would show a positive result for the presence of alkaloids.

Inhibitory potential of Garlic Aqueous decoction against identified pathogens

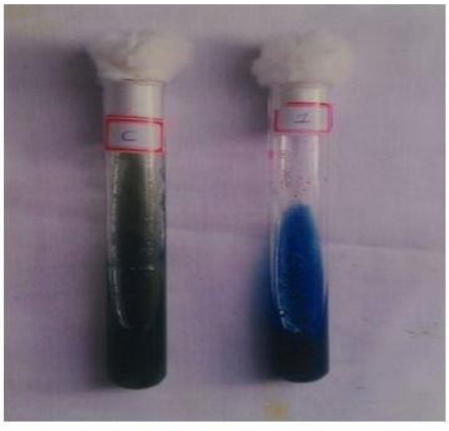
The antibacterial activity of the crude extracts was determined in accordance with the agar-well diffusion method described Eighteen-hour broth cultures were used for the study. The molten sterile nutrient agar (20 ml) was poured into sterile petri dish and allowed to set. The sterile nutrient agar plates were flooded with 0.2 ml of the standardized inoculum. A sterile corn borer (No. 6) was used to bore equidistant cups into the agar plate. One drop of the molten agar was used to seal the bottom of the bored hole, so that the extract will not sip beneath the agar. Different concentrations of decpotion (50 µg/ml, 100 µg/ml and 200 µg/ml) were introduced into the wells. A control was prepared by putting 50 µl of freshly prepared sterile distilled water in one of bored hole at the plates containing aqueous suspensions. One-hour pre-diffusion time was allowed, after which the plates were incubated at 37°C for 18 h.

RESULT:

From all collected cold food samples, four different pathogenic micro organisms were isolated and identified. Based on the staining technology morphological examination and identification has been done. Results were tabulated in Table 1. Mainly gram-negative organisms causing high level of food born infections. The major opportunistic organism, S. aureus were also identified. It might be due to the non-hygienic handling of food materials. All previous reports supporting the prevalence of gram-negative organisms and occurance of S. aureus in contaminating food samples and making health hazards[5].

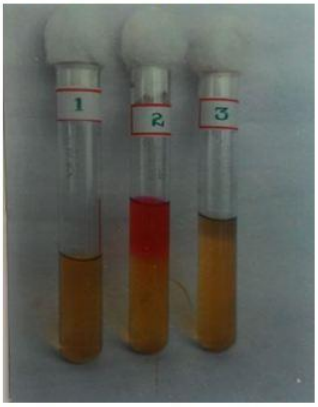
After morphological identification, all isolates were subjected for the biochemical charecterisation. Standard protocols were followed to perform biochemical analysis of isolates. Results were tabulated in table 2. Isolates were confirmed their identity based on the growth in culture specific media. It has been listed out in Table 3 and Fig. 1.

INDOLE TEST

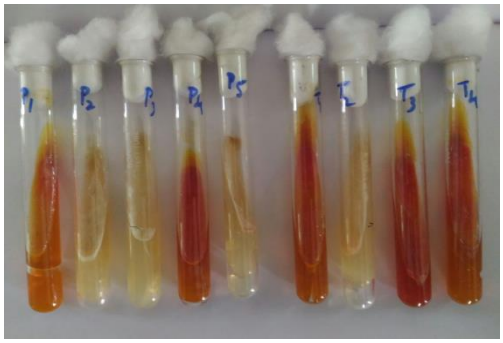


1.Positive (E.Coli) 2.Negative

METHYL RED TEST

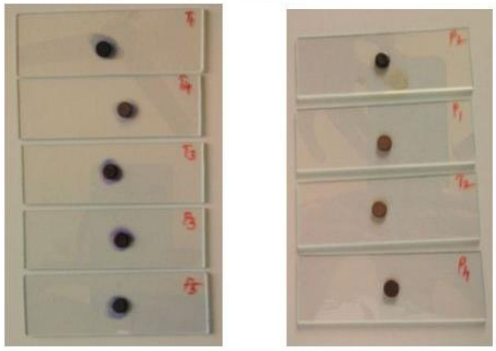


1.Control 2.Positive (E.coli) 3.Negative



Citrate Utilization test

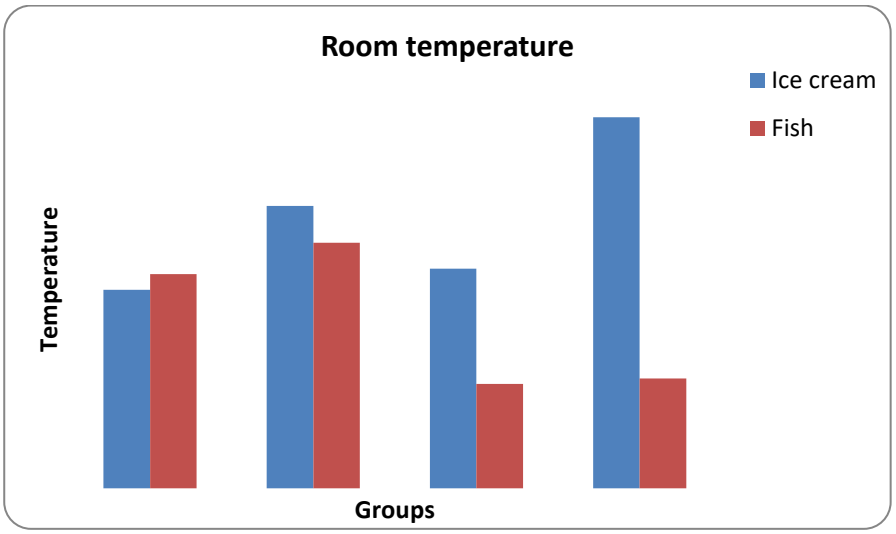
Triple sugar iron agar test



Oxidase test

Figure-1: Indiole test, Methul Red Test, Citrate utilization test, Triple Suger iron test.

Each isolate were subjected for growth kinetics study under different temeperature level. Since samples are coming under the category of cold storage food, it is necessary to find out the impact of different temperature in the microbial load. With this concept growth rate was measured under three different temperature such as -2 ^{0}C, 37^{0}C and 50^{0}C. OD values were taken and made graphical representation of each isolates (Fig.2)



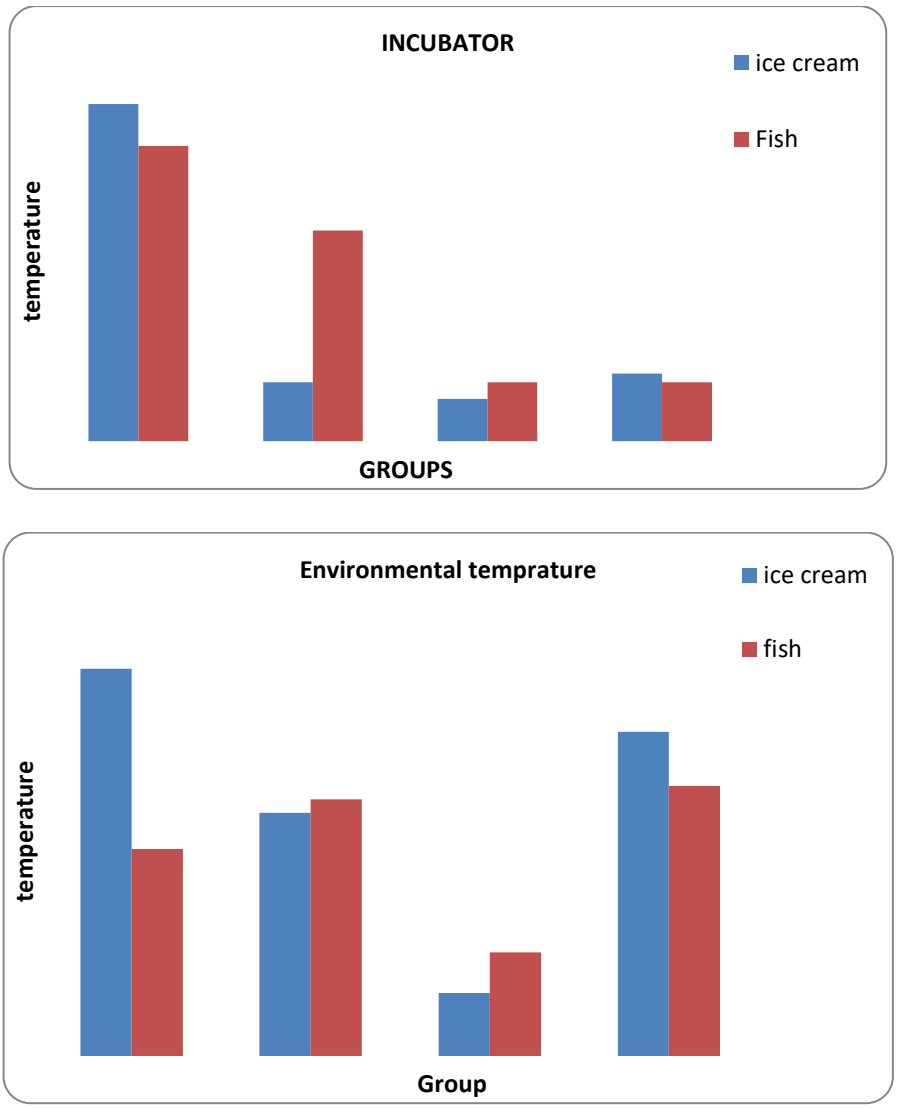


Figure-2: Graphical representation of room Temperature, incubator and Environmental temperature.

Based on the preliminary identification and Biochemical characterization of isolates, pathogenicity of each microorganism were stated. Its action against the major antibiotics (Erythromycin (15mg), kanamycin(30mg) and Chloramphenicol (30)) were tested. Many previous findings support the multiple drug resistant nature of S. aureus. Current test also showed the existence of MDR S. aureus as well as resistance of E. coli against kanamycin and Chloramphenicol. Different concentration of antibiotics showed different level od inhibition action against tested micro organisms. All results were supported by the earlier findings and tabulated in Table 4. Zone of inhibitions were presented in Fig.3

ANTI BIOTIC SENSITIVITY TEST

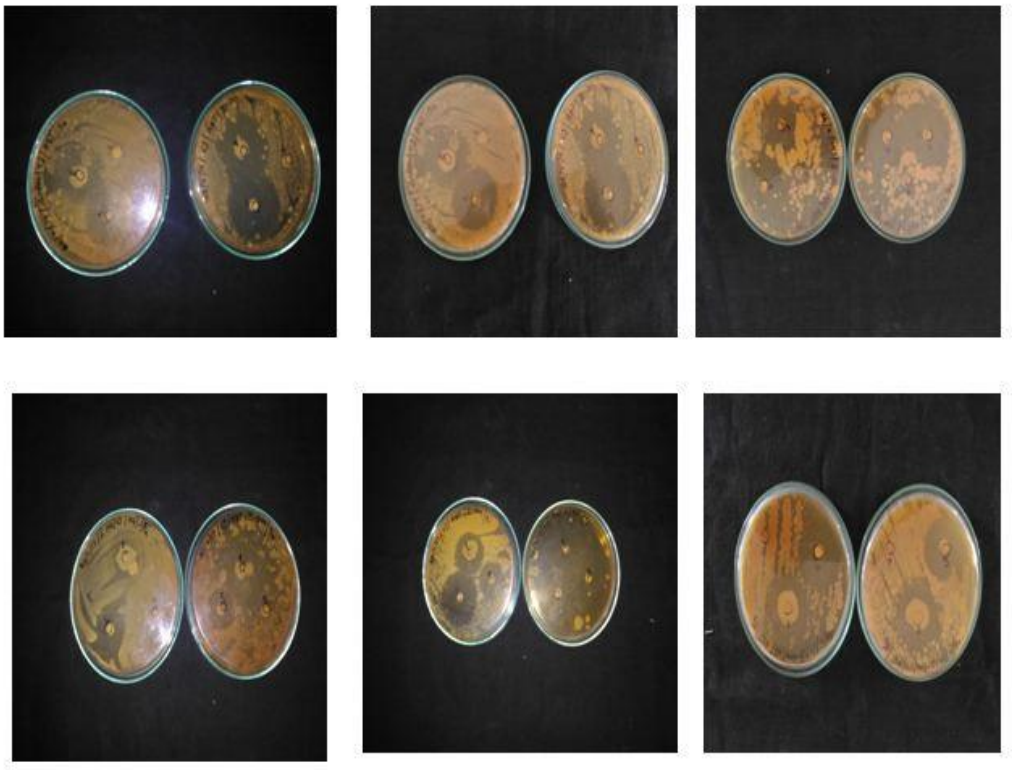


Figure-3: Representation of Anti Biotic Sensitivity Test

Aqueous decoction was used to evaluate the therapeutic potential of *Allium sativum* (Garlic). All the major phytochemicals were screened using standard protocols. Results showed the presence of major phytoconstituents such as alkaloids, triterpenes, steroids and glycosides. The screening results were tabulated in Table 5.

The aqueous decoction of Allium sativum was investigated for their antimicrobial activity against various food origin pathogens (*E. coli, Bacillus, S. aureus, Klebsilia*). The diffusion method was used in testing various concentrations of this decoction. The results depicted in Table 5 indicate that the high concentrations of Allium sativum aqueous extract (200 µg/ml) used in this study had the highest inhibitory effects (4.2mm) against the tested microorganisms. However, this extract showed inhibition action of 0.8mm even at minimal concentration (50 µg/ml) used in this study. The other concentrations of the aqueous phase (100 µg/ml) gave an inhibition zone of 3.6 and 1.6mm, respectively. All results were recorded (Table 4 and Fig. 4).

NATURAL SOURCES OF GARLICE TEST

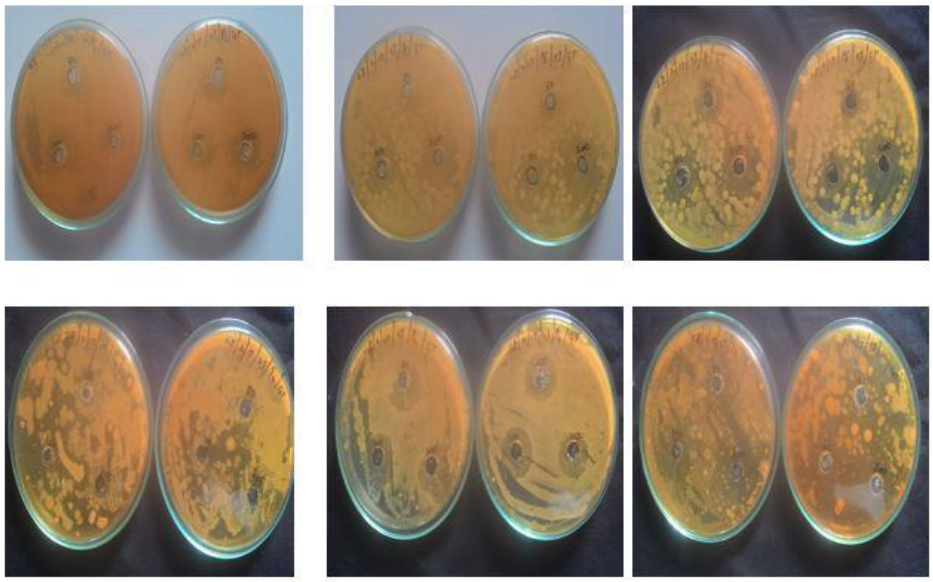


Figure-4: Representation of Natural source of Garlic test

Table 1: Morphological Identification Of Isolate

S.No	Isolates	Shape	The Colonies Observed	Type (+Ve/-Ve)	Souece
1	T1	Cocci	one	Both present	ICE
2	T2	Bacilli	two	Both present	ICE
3	T3	Cocci	Three colonies	Gram negetive	ICE
4	T4	Irreugular	Unknown	Gram negetive	ICE
5	Sfw I	Cocci	Observed	Gram negetive	FISH
6	Sfw II	Irreugular rhizoid	unknown	Both present	FISH
7	Sfw III	Cocci	Observed	Gram negetive	FISH
8	Sfw IV	Bacilli	Two	Both present	FISH

Table 2: Biochemical Characterization Of Isolates

S.no	Test	T1	T2	T3	T4	SF-I	SF-II	SF-III	SF-IV	SF-V
1	CATALASE TEST	-	-	-	+	+	_	_	+	+
2	OXIDASE TEST	+	-	+	+	-	-	+	-	+
3	UREASE TEST	-	+	+	-	+	-	-	+	-
4	TRIPLE SUGAR IRON TEST	+	+	+	_	_	+	+	_	_
5	ENDOLE TEST	+	+	+	+	-	-	+	-	-
6	MR-TEST	-	-	+	+	-	+	-	+	_
7	VP-TEST	+	+	-	-	_	+	_	+	+
8	CLTRATE UTILIZATION TEST	+	+	_	_	_	+	+	+	+

T1= ice cream local (Powdered drink),
T2=ice cream local (Coolers)
T3=ice cream local (Butter)
T4=ice cream local (Dairy)

fish-I =Mettur
fish-II =Bavani
fish-III =Pallipalaiyam
fish-V =Karur

Table 3: Identified Isolates

S.no	Isolates	Name of organisms	Source
1	T1	*E.coli*	Ice cream
2	T2	*Klebsilla*	Ice cream
3	T3	*Bacillus*	Ice cream
4	T4	*Staphylococcus*	Ice cream
5	Fish	*E.coli*	Fish water
6	Fish	*Klebsilla*	Fish water
7	Fish	*Bacillus*	Fish water
8	Fish	*Staphylococcus*	Fish water

Table 4: Antibiotic Sensitivity Test

S.NO	NAME OF THE MICROORGANISMS	ANTIBIOTIC	ZONE OF DIAMETR(mm)	SOURSE
1	*E.coli*	Erythromycin	1.3	Food
		Kanamycin	-	
		Chloromphenical	-	
2	*Klebsilla*	Erythromycin	1.2	Food
		Kanamycin	-	
		Chloromphenical	3.3	
3	*Staphylococcus*	Erythromycin	-	Food
		Kanamycin	-	
		Chloromphenical	-	
4	*E.coli*	Erythromycin	3.3	Fish
		Kanamycin	1.9	
		Chloromphenical	2.3	
5	*Klebsilla*	Erythromycin	2.9	Fish
		Kanamycin	2.6	
		Chloromphenical	3.5	
6	*Staphylococcus*	Erythromycin	-	Food
		Kanamycin	-	
		Chloromphenical	-	
7	*E.coli*	Erythromycin	3.7	Fish
		Kanamycin	-	
		Chloromphenical	3.1	
8	*Klebsilla*	Erythromycin	3	Fish
		Kanamycin	2	
		Chloromphenical	3.3	
9	*Staphylococcus*	Erythromycin	1.2	Food
		Kanamycin	-	
		Chloromphenical	1.6	

Table 5: Inhibitory Effect of Garlic Against Isolates

S.NO	Name of the microorganisms	Garlic (concentraion)	Zone of diametr (mm)	SOURSE
1	E.coli	A-200ml	-	Fish
		B-100ml	-	
		C-50ml	-	
2	Klebsilla	A-200ml	3.2	Fish
		B-100ml	2.3	
		C-50ml	1.6	
3	Staphylococcus	A-200ml	2.2	Food
		B-100ml	2	
		c-50ml	3.4	
4	E.coli	A-200ml	-	Fish

S.NO	Name of the microorganisms	Garlic (concentraion)	Zone of diametr (mm)	SOURSE
		B-100ml	-	
		C-50ml	-	
		A-200ml	1.8	
5	Klebsilla	B-100ml	1.6	Food
		C-50ml	2.3	
		A-200ml	-	
6	Staphylococcus	B-100ml	-	Fish
		C-50ml	-	
		A-200ml	2.6	
7	E.coli	B-100ml	2.4	Fish
		C-50ml	2.2	
		A-200ml	1.9	
8	Klebsilla	B-100ml	2.6	Food
		C-50ml	2.3	
		A-200ml	2.7	
9	Staphylococcus	B-100ml	2.4	Food
		C-50ml	1.3	

DISCUSSION

Present study stated the importance of hygienic handling in the preparation of various cold storage foods. From the tested ice cream as well as fish samples, a list of pathogenic micro organisms were identified and isolated. The occurrence of highly pathogenic *E. coli, Klebsilla, S. aureus* and *Bacillus* were noted. Three major antibiotic s were used to check the drug resistant nature of the identified isolates. Results showed the incidence of multiple drug resistant organisms. This is a highly hazardous signal for health conditions in human after having these foods.

Different levels of temperature conditions were settled to check the growth conditions of the micro organisms. Results showed the persistence of growth rate in all temperature levels. In frozen condition growth was limited. But when it was exposed to the room temperature, growth become highly active. In the higher temperature, growth level was in little limited level. Graph shows clear variation ranges in the growth of each organisms.

Since micro organisms shown resistance towards the major antibiotics, it became a necessity to find an option to resist the pathogens. The study was planned with a common culinary material "*Allium sativum*". Presence of major phytochemicals were noted after the preliminary screening. Only sterilized water decotion were used for the analysis. With this aqueous decoction, it showed high level inhibitory action against all micro organisms. Even the microbes which showed resistance towards the antibiotics also become sensitive towards Garlic decotion. This might be due the presence of major enzyme *Allicin* in Garlic.

CONCLUSION:

From this study, it can be concluded that common spice, *Allium sativum* (Garlic) can be a better option to find out a new drug material which can act against all kind of MDR organisms. Current study also suggests the importance of hygienic handling and preparation of food materials to avoid high level of health hazards among humans.

To characterize the particular compound which is responsible for this antimicrobial potential, more molecular levels studies may needed. It will be carried out in the future research.

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