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MICROBIOLOGICAL ANALYSIS OF FAST FOODS AND EVALUATION OF ANTIMICROBIAL ACTIVITY OF ALOE VERA GEL EXTRACT ON MICROBIAL PATHOGENS

R. Lakshmi [1*] and Karthika[2]

1*Associate Professor, Department of Microbiology, Justice Basheer Ahmed Sayeed College for Women, Chennai-18.

2 Department of Microbiology, Justice Basheer Ahmed Sayeed College for Women, Chennai-18.

*** Corresponding author email: laksh1573@gmail.com**

Abstract:

The present study was undertaken to assess the microbial quality of fast foods popular among students and to evaluate the antimicrobial activity of Aloe vera gel extract on the bacterial pathogens isolated from fast foods. Samples belonging to five different varieties of fast foods namely, Panipuri, Bhelpuri, Maggi, Pasta and Sevpuri, were obtained from eateries in and around educational institutions in Chennai City. They were processed and examined for the presence of pathogenic bacteria. *Escherichia coli, Pseudomonas aeruginosa, Bacillus spp., Staphylococcus aureus* and *Salmonella typhi were* isolated from the food samples. The antimicrobial activity of Aloe vera gel extract against the isolates was evaluated by Agar well diffusion assay. Comparative study was done using Antibiotics, namely, Meropenem, Erythromycin, Cefoperazone, Ciprofloxacin and Doxycycline. Aloe vera gel extract showed high efficacy towards *Escherichia coli* (15mm) followed by *Salmonella typhi (*14mm), *Bacillus* spp. (13mm), *Staphylococcus aureus* (9mm) and no zone of inhibition against *Pseudomonas aeruginosa*. This study emphasizes the importance of identifying natural products with promising antibacterial activity as a herbal alternative to Antibiotics.

Introduction:

Fast food is a kind of food which is bulk-produced and is provided in a short period of time. In comparison to other foods and dishes, this has a lower nutritive content. A dish with a short preparation time could be classified as fast food. The word

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usually refers to food provided in a restaurant or store which contains warmed or precooked materials and is provided to the consumer in a packed form for take-out/take-away. Fast foods, according to the National Institute of Health (NIH), are swift substitutes for home-cooked meals. Fast foods are rich in saturated fat, sugar, salt, and calories (Fernández & Juan, 2000).

Conventional street cuisine is sold all over the world by smaller, individual sellers who operate from either a wagon, table, mobile grill, or vehicle. Commonly, street vendors provide a colorful and varying range of options designed to quickly captivate passers-by and attract as much attention as possible. Food handling professionals, according to WHO (1989), play a significant role in maintaining safety of food all through the food manufacturing and storage chain. Improper processing and a lack of sanitary procedures by food handlers can allow harmful bacteria entering food, and these bacteria can live and grow in sufficient quantities to induce sickness in the consumer. Due to inadequate hygienic practices or cross-contamination, fast-food personnels' hands could act as vectors in the transmission of food-borne infections. Bacterial as well as other infectious agents could spread more readily in a setting where food is stored, handled, and prepared in an unhygienic manner. Food poisoning episodes could also be caused by insufficient temperature and time management, as well as cross contamination. Food - borne illnesses are commonly caused due to poor hygienic practices, indicating that food handlers' awareness and management procedures have to be enhanced. The prevention of disease transmission between food service workers to customers begins with proper personal hygienic and food preparation procedures (Kibret & Abera, 2012).

Materials and methods:

Food samples belonging to five different varieties of fast food were collected from different locations in and around educational institutions in Chennai.

Collection of fast-food samples

The samples were collected from the shops and immediately transferred to sterile containers. The samples were kept in an ice pack to prevent any changes in the microbial

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flora of the samples. The samples were transported to the laboratory for the analysis within 6 hours of collection.

Isolation and identification of pathogenic bacteria

25 g of homogenized sample was added to 225 ml of nutrient broth and then it was incubated at 37°C for 24 hrs. They were inoculated onto Nutrient agar, Eosin Methylene Blue agar, Cetrimide agar, Mannitol Salt agar and Deoxycholate Citrate agar plates. After incubation, the plates were observed for characteristic colonies and the bacteria were identified as per the standard protocol.

Evaluation of antimicrobial activity of Aloe vera gel extract on microbial pathogens

Extraction of gel from Aloe vera leaves

The fully expanded leaves of Aloe vera were selected from plants, washed with distilled water and were subjected to surface sterilization. The gel was drained out by peeling the parenchymatous covering of the leaves. Slurry was formed with the help of pestle and mortar; leaf gel was dried in the oven at 80°C for 48 hrs and then powdered. 10 grams of this powder was soaked in 100 ml of ethanol for 24 hours. The contents were filtered through Whatman filter paper No.1 and the filtrate evaporated to dryness. This dried extract was further powdered and then dissolved in distilled water in order to obtain the working solution with 10 mg/ml concentration.

Agar well diffusion assay using Aloe Vera gel extracts

Antimicrobial activity of Aloe vera gel extract against the isolated strains was evaluated by agar well diffusion assay. A suspension consisting of 100µl of each bacterial isolate was added to 100 ml of sterile Mueller Hinton Agar (MHA). It was mixed well and poured into sterile petri plates and was allowed to solidify. Wells of size 6mm were made on the solidified media with the help of a well puncture. 100µl of the Aloe Vera gel extract solution was pipetted into the wells of an assay plate. These plates were incubated at 37^0C for 24 hrs. Following incubation, petri-plates were observed for the inhibition zones. The diameter of the zone of inhibition was measured for each isolate and was compared with the control (Rudrangshu *et al*., 2015).

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Agar well diffusion assay using antibiotics

Antimicrobial activity of commercial antibiotics against the pathogenic strains was evaluated using agar well diffusion method. The antibiotics used for the study were as follows; Erythromycin (250mg), Meropenem (1g), Cefaperazone (1g), Doxycycline (100mg), and Ciprofloxacin (500 mg).

Working solution of 10mg/ml concentration of each antibiotic was used for the study. 100µl of the isolated strains were added onto 100 ml of sterile MHA, mixed well and poured into sterile petri plates. These plates were allowed to solidify and then marked respectively. Each plate was punched to make wells. 100µl of respective antibiotic solutions were pipetted into the wells in the assay plate. Plates were incubated 37^0C for 24 hrs. Following incubation, petri-plates were observed for the inhibition zones. The diameter of the zone of inhibition was measured for each isolate and compared with the control (Bauer *et al*.,1966).

Results:

Food samples from five different varieties of fast food namely Panipuri, Bhelpuri, Maggi, Pasta and Sevpuri were collected from different locations in and around educational institutions in Chennai. The samples were immediately transferred to sterile containers and were processed. A total of eight isolates were obtained. The isolates were identified based on preliminary tests and colony morphology on selective media and confirmed by biochemical characterization (Table 1).

Table 1 Showing prevalence of pathogens in the fast-food samples.

S.NO	SAMPLES	PATHOGENS ISOLATED
1.	Panipuri	*Escherichia coli*
2.	Bhelpuri	*Pseudomonas aeruginosa*
3.	Maggi	*Escherichia coli, Bacillus* spp.

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4.	Pasta	*Pseudomonas aeruginosa, Staphylococcus aureus*
5.	Sevpuri	*Salmonella typhi, Escherichia coli*

It was observed that *Escherichia coli* was prominently present in Panipuri, Maggi and Sevpuri samples. *Pseudomonas aeruginosa* was found to be present in Bhelpuri and Pasta samples, whereas *Bacillus spp* and *Salmonella typhi* were present in Maggi and Sevpuri respectively. *Staphylococcus aureus* was found to be present in Pasta.

Evaluation of antimicrobial efficacy of aloe vera gel extract on microbial pathogens

Agar well diffusion assay using aloe vera gel extract

The isolated strains were evaluated for the antimicrobial activity of Aloe Vera gel extract by Agar Well Diffusion Technique. The antibacterial property of Aloe Vera gel extracted using Ethanol showed varying degree of response towards the isolated organisms (Table 2).

Table 2: Showing Zone of inhibition of pathogens against Aloe vera gel extract

S.No	Isolate	Zone of inhibition(mm) against Aloe Vera gel extract
1.	*Escherichia coli*	15
2.	*Pseudomonas aeruginosa*	0
3.	*Bacillus species*	13
4.	*Staphylococcus aureus*	9
5.	*Salmonella typhi*	14

The above table shows that *Escherichia coli* was highly sensitive to the Aloe Vera gel extract showing a zone of inhibition of 15mm, followed by *Salmonella typhi* showing

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14mm, *Bacillus species* showing 13mm and *Staphylococcus aureus* showing 9mm. There was no zone observed with *Pseudomonas aeruginosa* indicating its resistance.

Agar well diffusion assay using antibiotics

The isolated strains were evaluated for the antimicrobial activity of antibiotics by Agar well diffusion technique. The antibiotics used were Meropenem, Erythromycin, Cefoperazone, Ciprofloxacin and Doxycycline. The zone of inhibition was observed for the antibiotics against the isolated organisms and this was compared with that of Aloe vera gel extract (Table 3).

Table 3 Showing Zone of inhibition against antibiotics

Isolates	**Zone of inhibition (in mm) against antibiotics**				
	Meropenem	**Erythromycin**	**Cefoperazone**	**Ciprofloxacin**	**Doxycycline**
E. coli	0	15	27	0	20
P. aeruginosa	0	0	23	0	18
Bacillus spp	0	0	0	0	0
S. aureus	0	0	0	0	0
S. typhi	26	0	20	27	20

The above table shows that *Escherichia coli* was highly sensitive to Cefoperazone (27mm) followed by Doxycycline (20mm) and Erythromycin (15mm), *Pseudomonas aeruginosa* was found to be sensitive to Cefoperazone (23mm) and Doxycycline (18mm), *Salmonella typhi* was sensitive to Ciprofloxacin (27mm), Meropenem (26mm), Cefoperazone and Doxycycline (20mm). *Bacillus spp* and *Staphylococcus aureus* showed no zone of inhibition to the antibiotics.

Discussion:

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Fast-foods are ready-to-eat foods sold by vendors especially in streets and other similar places. These fast foods provide an affordable source of nutrients to the population who appreciate the food due to its taste, low price and availability at the right time. Food-borne illnesses are often associated with the consumption of street-vended fast foods, restaurants and homemade kitchen food.

In the present study, microbiological analysis of five varieties of commonly available fast-food samples collected from different locations in and around educational institutions in Chennai was done. The samples showed the presence of *Escherichia coli*, *Pseudomonas aeruginosa, Bacillus spp, Staphylococcus aureus* and *Salmonella typhi*. *Escherichia coli* was predominantly present in almost all the samples, followed by *Pseudomonas aeruginosa*, *Bacillus spp, Staphylococcus aureu*s and *Salmonella typhi.*

A similar study was conducted by Rudrangshu *et al*., (2015), on Microbiological Analysis of fast-food samples collected from Delhi Suburb. The samples analyzed were Spring roll, Chat, Panipuri, Burger, Sandwich, Bhelpuri, Pav bhaji and Bread Pakora. The pathogens identified from their study were *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, and *Staphylococcus aureus*. It was observed that *E.coli, Bacillus cereus* and *Staphylococcus aureus* were predominantly present. However, *Pseudomonas aeruginosa* were found to be present in Chat, Panipuri and Bhelpuri.

In the present study, extraction of gel from Aloe Vera using ethanol and the evaluation of susceptibility and resistance patterns of the bacterial isolates against the Aloe vera gel extract was done by agar well diffusion method using Muller Hinton Agar. The antibacterial property of Aloe Vera gel extract using Ethanol showed varying degree of response towards the isolates. The zone of inhibition against Aloe Vera gel extract showed its high efficacy towards *Escherichia coli* (15mm) followed by *Salmonella typhi* (14mm), *Bacillus spp*., (13mm), *Staphylococcus aureus* (9mm), and no zone of inhibition was found against *Pseudomonas aeruginosa*.

These results justify the popular use of Aloe Vera gel to relieve many types of gastrointestinal irritations (Foster, 1999; Grindlay & Reynolds, 1986). The gel promotes

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wound healing due to the presence of components like anthraquinones and other components like lupeol, cinnamic acid, salicylic acid, urea, phenols and sulfur (Ernst, 1998) possess antibacterial, antifungal and antiviral activities. In the present study, Aloe Vera gel was found to possess 100% inhibitory effect on Gram positive bacteria.

Rudrangshu *et al*. (2015), also studied the antibacterial property of Aloe Vera gel extracts obtained using Ethanol, on bacterial pathogens. The Zone of Inhibition against Aloe Vera gel extract showed high sensitivity towards *Staphylococcus aureus* (22mm) followed by *Bacillus cereus* (20mm), *Escherichia coli* (14mm) and *Pseudomonas aeruginosa* (12mm). Olaleye & Michael (2005) and Reynolds & Dweck (1999) have also reported antibacterial properties of ethanol extracts of Aloe Vera gel against pathogens and no antimicrobial activity was reported using aqueous extract of Aloe Vera leaves (Martinez *et al*., 1996).

In the present study, the antimicrobial efficacy of Aloe Vera gel extract with that of commercial antibiotics was compared. *Escherichia coli* was highly sensitive to the Aloe Vera gel extract showing a zone of inhibition of 15mm, followed by *Salmonella typhi* showing 14mm, *Bacillus species* showing 13mm, *Staphylococcus aureus* showing 9mm. There was no zone observed with *Pseudomonas aeruginosa* indicating its resistance.

The antibiotics used in the present study were Meropenem, Erythromycin, Cefoperazone, Ciprofloxacin and Doxycycline. *Escherichia coli* was found to be sensitive to Cefaperazone, Doxycycline and Erythromycin, *Pseudomonas aeruginosa* was found to be sensitive to Cefaperazone and Doxycycline and *Salmonella typhi* was found to be sensitive to Ciprofloxacin, Meropenem, Cefaperazone and Doxycycline. *Bacillus* spp and *Staphylococcus aureus* showed no activity against the antibiotics.

A similar study by Rudrangshu *et al*. (2015), showed *Escherichia coli* to be sensitive to Meropenem, Cefaperazone and Ciprofloxacin, *Pseudomonas aeruginosa* to be sensitive to Meropenem, Cefaperazone and Ciprofloxacin, *Bacillus cereus* to be sensitive to Meropenem, Cefaperazone, Erythromycin and Ciprofloxacin and *Staphylococcus aureus* to

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be sensitive to Meropenem, Erythromycin and Cefaperazone and all isolates showed high level of resistance to Doxycycline.

The results of our study differ in the activities of Meropenem which was found to have effect only on *Salmonella typhi* and no activity against any of the other isolates. Doxycycline was found to be effective against *Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi* which again contradict the results of Rudrangshu *et al*. (2015).

The fast increase in the appearance of multi-drug-resistant bacteria is a major problem faced by medicine and science today. Antimicrobial resistance in the bacterial population has increased worldwide and its susceptibility patterns show substantial geographic variation as well as differences in population and environment. The presence of multi-drug-resistant strains is alarming, because such strains lead to a higher fatality rate than sensitive ones. Obviously, the prudent use of antimicrobial agents is a pre-requisite for the minimization of the emergence of resistant bacteria, but such prudence in itself is not enough to control this emerging public health threat.

Conclusion:

The widespread emergence of resistance among microorganisms against available antibiotics is a major concern. The present study has revealed the importance of natural products to control antibiotic resistant bacteria, which are a threat to human health. As global antibiotic resistance in bacteria is becoming a public health concern and the race to discover the new antibacterial agent is on, Aloe vera gel along with its identified compounds with promising antibacterial activity could be used as an alternative herbal remedy. Hence, it can be concluded that the compounds isolated from Aloe vera gel extracts could be recommended for further trials to determine the proper dosage against different bacterial pathogens. At the same time, this preliminary study could further lead to the identification of alternate drugs to control the multi-drug-resistant bacterial strains.

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