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Isolation and Identification of food borne pathogens from Spoiled food samples

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A B S T R A C T

Studies were performed to identify various pathogenic bacteria from different sources like; spoiled fruits, vegetables, Dairy products, Bakery products, Poultry products and Spoiled rice were selected from local market of Bagalkot district, Karanataka, India. Collected samples were examined in specific media like Mannitol Salt agar, MacConkey agar and Cetrimide agar. Samples were confirmed by both phenotypic and genotypic characteristics. The pathogenic bacterial isolates such as *Pseudomonas* spp [23.66%], *Staphylococcus aureus* [22.76%], *Salmonella* spp [21.87%], *E.coli* [22.32%] and *Klebsiella* spp [8.92%] were present in large amount. The percentage of multidrug-resistant (MDR) isolates was high in numbers and the most prevalent resistant pattern included four different classes of antibiotics; Cephalosporins, Fluoroquinolones, Beta-Lactams and Aminoglycosides.

Introduction

Microorganisms are the primary cause of food spoilage and food borne illness. Food borne diseases are globally important, as they result in considerable morbidity, mortality, and economic costs. Many different sources like bacteria, viruses, parasites, chemicals, and prisons, may be transmitted to humans by contaminated food. Outbreaks and sporadic cases of food borne disease are regular occurrences in all countries of the world (Kirk *et al*., 2015). However, the statistical data of food borne illness is increased due to the unrecognized or unreported outbreaks particularly in the developing countries (Han *et al*., 2014).

There has been a continuous increase in several food borne diseases caused by bacterial pathogens such as *Salmonella, Campylobacter, E.coli, Listeria, S.auerus, Klebsiella* and *Pseudomonas.* These pathogens come into contact with foods during harvest or slaughtering, processing, storage and packaging. Environmental challenges have caused food-borne bacterial pathogens to evolve and the susceptibility of human population to infections (Chibeu, 2013),Generally, food borne diseases are associated with acute, mild and self-limiting gastroenteritis with symptoms such as nausea, vomiting and diarrhea as a consequence of consumption of microbial contaminated food and number of chronic sequences may result from food borne

infections involving diseases that affect the cardiovascular, musculoskeletal, and respiratory and immune systems (Han *et al*., 2014).

Most bacteria and fungi that arrive on the developing crop plant like fruits and vegetables provide a natural biological barrier to infestation by the subset of microorganisms responsible for crop damage (Mohamed *et al*., 2012) when the soil is fertilized by human sewage or if crops are irrigated with sewage water and also during harvesting and post harvest handling, or during storage, distribution or also get contaminated by soil, air and water (Mohamed *et al*., 2012) Moreover, food products are at risk of contamination with mishandling during processing and preparations where there are favorable conditions for pathogens (Kakarla *et al*., 2015) and also responsible for causing spoilage to the edible portion of the crop. In most of the cases food like dairy product, fish and poultry product get contaminated while handling of the product, harvesting and processing equipment and transport (Saranraj *et al*., 2012) Food products may become contaminated at different stages along the food chain, from growth or production until the final consumption (Sillankorva *et al*., 2012).

In most of the cases microorganism like *E.coli* in meat product, drinking water and dairy product; *Salmonella*, *S.auerus* in meat, eggs, on vegetables and poultry; *Listeria* in dairy product and shellfish; *Pseudomonas* on fruit, dairy product, poultry and in drinking water.

The prevalence of Multi Drug Resistant (MDR) foodborne pathogens is increased by consumption of contaminated food because they are responsible for more serious disease than susceptible bacteria (Gohary *et al*., 2015). Antibiotic is ineffective due to bacterial resistance, and therefore the infection persists and the illness progresses (Brunelle *et al.,* 2013). The main objective of this study is Identification of pathogens from different food source like Fruits, Vegetables, Dairy products, Bakery products, spoiled rice and Poultry product collected from the bagalkot district.

Materials and Methods

Sample collection

A Total of 79 different food sources are collected from local market in and around Bagalkot district, Karnataka, India. All the samples were wrapped separately in sterile polyethylene bags and plastic bags and transported to the laboratory for microbial analysis.

Isolation of food borne pathogens

The samples are rinsed thoroughly with distilled water and used for isolation of bacteria on specific media Mannitol Salt agar, MacConkey agar and Cetrimide agar at 37°C for 24 hours.

Phenotypic characterization of isolates

Bacteria are identified by cultural characteristics such as abundance of growth, color change in media and morphological characteristics like form, size, margin and elevation are studied on culture plates. Identifying an isolates by Gram's reaction-Gram's staining, Motility determination-Hanging drop method and Soft agar stabbing (Tube Method), Catalase, Oxidase, Nitrate Reduction, IMVIC test, Carbohydrate Utilization, Urease production, Gelatin Hydrolysis, Coagulase Test and DNase Test were performed for the confirmation of the Bacterial isolates according to the bergey's manual.

Antibiotic Susceptibility Test

Antimicrobial Susceptibility testing was performed on Mueller- Hinton Agar by Standard disk diffusion method as recommended by the CLSI-2010. The twelve different antibiotic discs were used like, Cefotaxime (cephotaxime) (30mcg), Nitrofurantoion(300mcg), Co-trimoxazole (Sulpha/trimethoprim) (25mcg), Meropenem (10mcg), Imipenem (10mcg), Amikacin (30mcg), Gentamicin (10mcg), Tetracycline (30mcg), Amoxyclav (30mcg), Ertapenem (10mcg), Ceftriaxone (10mcg) and Cefazolin (30mcg). Mueller-Hinton plates were incubated at 35◦C for 16 to 18 hours after inoculation with organisms and zones of inhibition were measured in Millimetre (mm).

Determination of Minimal inhibitory concentration (MIC)

Determination of resistant isolates was used for MIC by agar dilution method. MIC was done by using Meropenem, Amikacin, Amoxyclav and Gentamicin antibiotic with different concentration.

Results and Discussion

Sample collection

Samples are collected from local market in and around Bagalkot district, Karanataka, India, in sterile plastic bags and microbial analysis are done for those samples as shown in table 1.

Isolation of food borne pathogens

Pathogens are isolated by using specific media like 1)Mannitol Salt agar was used for the identification of food borne *Staphylococcus aureus* which ferment Mannitol, whereas *Staphylococcus epidermidis is a* non fermenting Mannitol. 2) MacConkey Agar was used for the identification of food borne *Salmonella* which is non-Lactose fermenter and Lactose fermenting organism like *E.coli* and *Klebsiella* and 3) Cetrimide agar was used for the identification of food borne *Pseudomonas* which is selective media for the bacterium. By using 3different media totally different organism were identified based on color change in media and colony morphology as shown in table 2. Among the various isolated pathogens *Pseudomonas* is predominant so, we are focusing on *Pseudomonas.*

Phenotypic characterization of isolates

Based on color change in media, morphological characteristics and biochemical characteristics were studied and confirmed. Out of 79 samples, 224 total isolates are found in present work, in that 53 *Pseudomonas* isolates [23.66%], 51 *Staphylococcus aureus*[22.76%], 49 *Salmonella*[21.87%], 50 *E.coli* [22.32%] and 20 *Klebsiella*[8.92%]. *Pseudomonas* is a Gram negative, rod shaped, Motile (Unipolar), Non spore forming, Catalase positive, Oxidative positive, Nitrate Reduction positive and Non-fermentative. *Staphylococcus aureus* is a Gram positive, cocci, Non-Motile, Non-Spore forming, Catalase positive, Nitrate Reduction positive, Methy-red positive, Voges-proskauer positive, DNase positive and Fermentative. *Salmonella* is a Gram negative, rod shaped bacteria, Non spore forming, predominately motile, Peritrichous flagella, Catalase positive, Nitrate Reduction positive and Fermentative. *E.coli* is a Gram negative, Non spore forming, rod shaped bacteria, Motile, Flagellated, Catalase positive, Nitrate Reduction positive and Fermentative. *Klebsiella* is a genus of non motile, Non spore forming, Gram negative, oxidase negative, rod shaped bacteria with prominent polysaccharide based capsule.

Antibiotic Susceptibility Test

In this test many of the pathogens shows resistance towards four different classes of antibiotic(as shown in fig 2) as recommended by the CLSI-2010(Clinical Laboratory Standard Institute) and Quality Assurance Guidelines(QAG) of WHO (World Health Organization).

Determination of Minimal Inhibitory Concentration (MIC)

MIC was carried out for all four antibiotics. Among total 53 *Pseudomonas* isolates, 21 isolates showed multidrug resistance. 20 resistant isolates were selected for MIC; the obtained results are shown in table 4.

The incidence of food borne illnesses is found to be increased day to day life. The presence of microorganisms is more in numbers of viable bacteria, an indicator of the expected shelf life of the food sources , increases the likelihood of spoilage, as well as the possibility of produce-associated outbreaks (Allydice-Francis *et al.*, 2012).

The microorganisms present in Fruits, Vegetables, Dairy products, Spoiled rice, Poultry products and Bakery products are a direct reflection of the sanitary quality of the cultivation water, harvesting, transportation, storage, and processing of the product (Eni *et al*., 2010).

At the present 79 samples were examined for bacterial growth in specific media and the bacterial isolates were identified and confirmed by Biochemical tests include 53 *Pseudomonas* isolates [23.66%], 51 *Staphylococcus aureus* [22.76%], 49 *Salmonella* [21.87%], 50 *E.coli* [22.32%] and 20 *Klebsiella* [8.92%].
Prevalence of common food borne pathogens (*Salmonella*, *Staphylococcus* and *E. Coli*) in chicken meat(Glenn *et al*.,2015) (Ruban *et al.*, 2012).Prevalence of *S. aureus* in many food products including raw retail meat indicating that consumers are at potential risk of *S. aureus* colonization and subsequent infection (Kadariya *et al.*, 2014). Consumption of raw or insufficiently heat treated fish, which may be contaminated with bacteria from water environment (*Vibrio* spp., *C. botulinum*) or terrestrial sources (*C. perfringens*, *Salmonella* spp., *Shigella* spp., *Staphylococcus* spp., *V. cholerae*), or fish products recontaminated after heat processing (Novotny *et al.*2004). *Salmonella*, *Escherichia coli*, and *Staphylococcus aureus* are the main predominant species in most food poisoning cases associated with contaminated raw or undercooked poultry and red meat (Gwida *et al*., 2015) (Dan *et al*., 2015). *Salmonella spp* and *Escherichia coli* are the two most important food-borne pathogens of public health interest incriminated in poultry meat worldwide (Adeyanju *et al*., 2014).

Pseudomonas spp. was one of the most frequently identified agents associated with waterborne outbreaks of dermatitis (rash or folliculitis), as well as conjunctivitis, otitis externa and other symptoms, in recreational water in the United States of America (Uğur *et al*.,2012)and shows resistance of *P. aeruginosa* to commonly used therapeutic agents has increased in recent years.

Multidrug-resistant (MDR) can be defined as resistance to at least four classes of antibiotics used during treatment of these infections: third-generation Cephalosporins, Fluoroquinolones, Aminoglycosides, and Carbapenems (Gómez *et al*., 2012). In present work *Pseudomonas*, *Staphylococcus aureus*, *Salmonella*, *E.coli* and *Klebsiella* shows resistance for four classes of antibiotics (Cephalosporins, Fluoro-quinolones, Beta-Lactams and Aminoglycosides).

Table.1 Number of samples used for isolation

Sl .no	Source	Samples	Total number of samples	Total number of isolates
1	Fruits	Apple(BG) I, Apple (S) II, Mosambi, Orange, Fig, Strawberry, Banana, Small banana, Chickoo, Passion fruit, Grapes (Black), Custard apple, Lemon, Musk melon, Javari banana and Water mellon.	16	43
2	Vegetables	Tomato (bg), Tomato (S), Bitter guard, Green chilly, Capsicum (B), Capsicum (S), Potato, Onion, Beans, Carrot, Ladies finger, Flat bean(surti papdililva), Methi leave (fenugreek greens), Coriander leaves, Amla, Ridge guard, Cauliflower, Gulgaya /meka, Spinach, Peace, Cluster beans, Pundi, Drum stick and Brinjal	24	70
3	Dairy products	Cow milk, Raw milk, Sambhrudi, Peda[nandini], Butter milk, Curd, Kunda[Belgaum], Cheese slice, Cheese cube, Shrikhand, Paneer and Dairy sewage.	13	45
4	Bakery products	Cake, Bread, Karadantu, Soan papdi, Ladagi ladu, Moothi chur, Gulab Jamun, Mysurpak, Badam puri, Besan laddu, Peda and Bella Jellaby.	12	38
5	Poultry products	Chicken, Mutton, Snake fish, Fish[M], Egg[Y] and Egg [Y+W]	12	22
6	Spoiled rice	Sample 1 & Sample 2	02	06
			Total No of samples=79	Total No of isolates=224

Table.2 Number of isolates in food samples

Sl .no	Sample	Total number of samples	Total number of isolates	Total number of *Pseudomonas* isolates	Total number of *S.auerus* isolates	Total number of *Salmonella* isolates	Total number of *E.coli* isolates	Total number of *Klebsiella* isolates
1	Fruits	16	43	06	11	12	09	05
2	Vegetables	24	70	21	18	14	14	03
3	Dairy products	13	45	12	06	09	11	08
4	Bakery products	12	38	03	12	10	09	04
5	Poultry products	12	22	10	04	03	05	00
6	Spoiled rice	02	06	01	00	01	02	00
		Total No of samples=79	Total No of isolates=224	Total no of *Pseudomonas*=53 [23.66%]	Total number of *S.auerus=51* [22.76%]	Total number of *Salmonella=49* [21.87%]	Total number of *E.coli=50* [22.32%]	Total number of *Klebsiella=20* [8.92%]

Table.3 Percentage of multi drug resistant bacteria as shown in table 3

Isolates	Antibiotics used for the Antibiotic Susceptibility Test											
	CTX %	NIT %	COT %	MRP %	IPM %	AK %	AMC %	GEN %	TE%	ETP %	CZ %	CTR %
Pseudomonas	45	43	65	77	52	65	97	84	46	54	46	40
S.auerus	77	45	46	52	43	97	54	65	84	65	46	54
Salmonella	84	54	43	65	45	52	77	46	97	46	65	46
E.coli	45	46	65	84	54	43	46	52	65	97	40	77
Klebsiella	65	43	40	46	97	54	84	77	46	52	45	65

Cefotaxime (cephotaxime)30mcg – [CTX], Nitrofurantoion 300mcg –[NIT], Co-trimoxazole (Sulpha/trimethoprim)25mcg –[COT], Meropenem 10mcg – [MRP], Imipenem 10mcg –[IPM], Amikacin 30mcg – [AK], Gentamicin 10mcg –[GEN], Tetracycline 30mcg –[TE], Amoxyclav 30mcg –[AMC], Ertapenem 10mcg – [ETP], Ceftriaxone 10mcg – [CTR], Cefazolin 30mcg – [CZ].

Table.4 MIC of the resistant bacteria

Sample	Antibiotics				Sample	Antibiotics			
	Meropenem	Amikacin	Amoxyclav	Gentamicin		Meropenem	Amikacin	Amoxyclav	Gentamicin
R1	32µg	8µg	1024µg	16µg	R11	128µg	64µg	1024µg	128µg
R2	16µg	8µg	256µg	8µg	R12	8µg	8µg	512µg	8µg
R3	16µg	8µg	512µg	8µg	R13	64µg	8µg	512µg	8µg
R4	16µg	8µg	512µg	8µg	R14	64µg	8µg	256µg	8µg
R5	32µg	8µg	512µg	8µg	R15	64µg	8µg	512µg	8µg
R6	8µg	8µg	512µg	8µg	R16	8µg	8µg	512µg	8µg
R7	8µg	8µg	64µg	8µg	R17	64µg	8µg	512µg	8µg
R8	64µg	8µg	512µg	8µg	R18	32µg	8µg	512µg	8µg
R9	8µg	8µg	512µg	8µg	R19	32µg	8µg	256µg	8µg
R10	8µg	8µg	512µg	8µg	R20	64µg	8µg	512µg	8µg

Fig.1 Growth of bacterial colonies on specific media

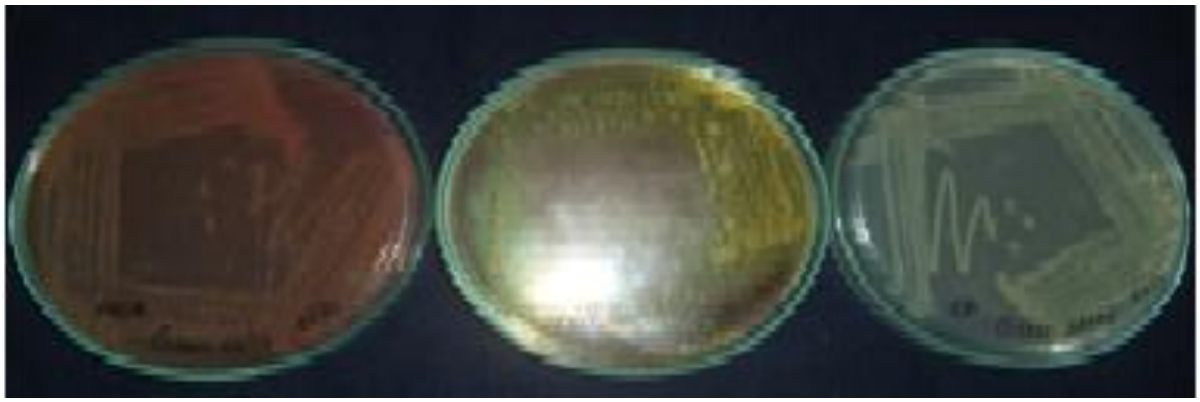
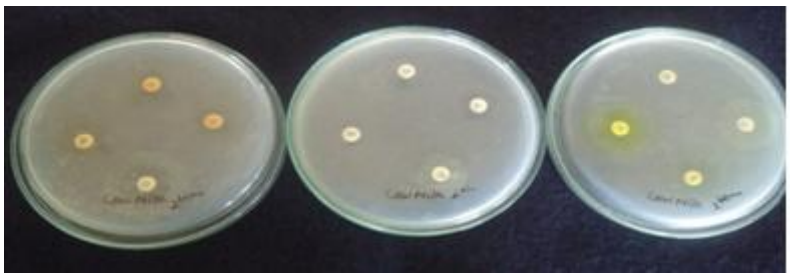


Fig.2 Pathogens showing resistance for Multi Drug



Resistance of food borne pathogens to antibiotics is a serious problem and the development of resistance-free antibacterial agents is necessary to treat bacterial infections effectively. In present work 20 multidrug resistant bacterial isolates were selected for Minimum Inhibitory Concentration (MIC) and results were as shown in table 3. Few bacteria show resistant at low concentration and few shows for higher concentration. These organisms have frequently been associated with food products and linked to a number of human illness cases from various products such as meat, milk and milk products, vegetables, poultry, and fish due to microorganisms resistant to multiple antimicrobial agents showed great antibacterial effectiveness on four important food borne pathogens.

In conclusion, prevalence of food borne illness and food borne pathogens were increasing day to day life. Due to food products get contaminated while handling, harvesting and processing in equipments and transportation. Food products may become contaminated at different stages along the food chain, from growth or production until reach to the consumers. Keeping all this in our mind we made a little effort to adders this serious concerned. In present work we have isolated over 79 food samples like spoiled Fruits, Vegetables, Dairy products, Bakery products, Poultry products and Spoiled rice were selected to isolate food spoiling bacteria. Among the isolates many of them shows resistance or had reduced susceptibilities to multiple antimicrobial agents. And also we found most of the isolates belong to *Pseudomonas spp*. It has shows high multi drug resistance rate compared to other bacterial isolates.

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