## Improper Storage of Grains: An Important Cause of Spread of Zoonotic Infections

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#### **ABSTRACT**

In order to learn the spread of zoonotic infections and its impact on human health we have enlighten one of the aspect i.e. improper storage of grains that are used in daily diet and because of economic lapses the shopkeepers and godown owner do not concern for high quality. The natural reservoirs of zoonose are wild life but now pets and domestic animals are also carrying zoonose and transmitting it to human population as wild life do. This study was performed to determine the amount of zoonotic infections spread by rodents through improper grain storage. Nine samples were collected from different go downs and grocery stores, of Federal B Area (water pump grocery market) and Gulshan e Iqbal Karachi. Each sample was processed for the presence of bacteria, actinomycetes, and fungal species. We found that grain samples did contain Gram positive strains i.e., Bacillus sp., Staphlococcus sp., Listeria sp., Clostridium sp. with few gram negative strains of bacteria. It is concluded that the improper storage of grains and prevalence of rodents in go downs and grocery shops contribute a lot in the spread of zoonosis, hence proper storage planning and ISO standardization should be followed appropriately to overcome the spread of zoonoses. The rodent diseases are fatal and it should be treated in time before complications, otherwise these rodents may spread a wide range of infections to human.

Keywords: Zoonotic infections, Grains, Rodents, Storage.

### INTRODUCTION\*

Emergence and re-emergence of zoonotic infections may be on certain factors of emergence, surveillance and control. Domestic animal like pets, livestock and wild life animal work as reservoir of infection that can be transmitted to humans (Kruse *et al.*, 2004). The significance of zoonose is being vastly increasing its socioeconomical impact which is not spreading only in under developing countries but also in developed countries, apart from causing human suffering, morbidity and mortality, they hamper agricultural production, decrease availability of food, and create barriers to international trade.

The increase interaction between animal and human population, trade of animals and animal product, illegal slaughtering, and inappropriate waste disposing are some factors increasing zoonoses worldwide (pandemic). Infestation of grain by arthropods belongs among the most economically important factor; since these pests cause enormous losses of stored products each season worldwide (Woolhouse and Gowtage-Sequeria, 2005; Meslin, 1992).

However, the direct impact on health of these new, emerging or re-emerging zoonoses has been small compared to that of other infectious diseases affecting humans (Wolfe *et al.*, 2005). Zoonoses can cause worldwide emergence of new diseases which may be mix with diagnoses of other human diseases, and lead to complications

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and consequence related to zoonoses. Although many zoonotic infection can be prevented and vaccinated and treated. All kinds of microbes have ability to cause zoonotic infections including bacteria, viruses, protozoal disease, and it can be the reasons involve in laboratory acquired infections, dealing with contaminated specimen with improper laboratory conditions and personnel skills. Although laboratory acquired infections are asymptomic but can be prevalent in future (Matschke and Faerstone, 1984). Food which is the basic source of energy for humans and animals is the major source of the spread of zoonoses. The improper storage of food contribute in the development of many zoonotic infection, zoonotic spread by rodents in the stored food in godowns is very common and the spread of rodent zoonose is increasing worldwide (Outbreak news, 2006). There are several ways to control these rodents base zoonoses. Although many factors other than rodents also involve in spoilage of food storage.

Strategies to improve public health have focused on improved surveillance in regions of perceived high likelihood of disease (reemergence). These strategies include improved detection of pathogens in reservoirs, early outbreak detection, broad-based research to identify factors that favor reemergence, and effective control (i.e., quarantine and improved hygiene) (Girard et al., 2004). However, control of zoonotic infections in reservoir hosts has a pronounced protective effect in human populations. Change in agricultural practices has become the dominant factor determining the conditions in which zoonotic pathogens evolve, spread, and eventually enter the human population. Livestock pathogens are subjected to pressures resulting from the production, processing and retail environment which together alter host contact rate, population size and/or microbial traffic flows in the food chain (Blancouet al., 2005).

Our basic aim of the study was to determine pathogenic microorganisms in improperly stored grains.

### **MATERIALS AND METHODS**

Collection of Samples: Samples were collected from different go downs and grocery stores, of Federal B Area (water pump grocery market) and Gulshan e Iqbal Karachi. Nine samples of waste grains, or grains spill through chewed or tore bags by rodents and nine sterile swab samples rubbed on the corners of shop and backwalls of the storage bags, where rodents penetration are suspected. The swabbed samples were placed in phosphate- bufferssaline (PBS) in glass test tube tightly covered till proceed. Two samples of feacal matter of rodents also collected and were also placed in phosphate- buffer -saline till proceeded.

Isolation of Microorganisms: Each sample was processed for the presence of bacteria, actinomycetes, and fungal species. Each of the grain samples weighing 1 gm was added in 2 ml of nutrient broth for bacterial growth. Similarly each of two feacal sample was placed in 2 ml of PBS and incubated for at least 2 days to enhance bacterial decomposition of feacal matter. Feacal matter was thoroughly mix and by wet mount technique was stained and observe under oil immersion lens. Selective culture media were used for the isolation and suspected colonies were re-streaked to obtain pure culture. For the selective isolation of actinomycetes from natural mixed microbial populations a nutrient agar medium was overlaid with a 0.22- to 0.45-microns membrane filter, and the surface of the filter was inoculated. During incubation, the branched mycelia of the actinomycetes penetrated the filter pores to the underlying agar medium, whereas growth of nonactinomycete bacteria was restricted to the filter surface. The membrane filter was removed, and the agar medium was re -incubated to allow the development of the isolated actinomycete colonies (Hirsch and Christensen, 1983).

by morphological, cultural and biochemical characteristics.

### $Identification\ and\ Characterization\ of\ Isolates:$

Isolates were identified and characterized

### **RESULTS**

**Table I.** Cultural characteristics of isolates from grain samples from different go downs and grocery shops.

Nutrient Agar	Manitol Salt Agar	Blood Agar	Sabouraud
	-		Dextrose Agar
Small white colonies	Manitolnon-fermenterpin	Not	Not Performed
	pointed colonies	Performed	
Small flat white	No change ,small white color	Not	White, opaque
colonies	mucoid colonies	Performed	small colonies
Small flat white	Yellow and pink colonies	Not	Not Performed
colonies		Performed	
Small flat white	Intense pink color colonies	Not	Not Performed
colonies		Performed	
Small flat white	yellow color colonies	Not	Not Performed
colonies		Performed	
Small flat white	Slightly pink color colonies	Not	Not Performed
colonies		Performed	
Small flat white color	Fungal growth observe along	Dark dull	Not Performed
colonies	with yellow color colonies	colonies, No	
	·	hemolysis	
Small flat white color	Yellow ,flat, dry colonies	Dark dull	Not Performed
colonies		colonies, No	
		hemolysis	
Small flat white color	Slightly yellow color colonies	Dark dull	Not Performed
colonies		colonies, No	
	Small white colonies  Small flat white colonies Small flat white colonies Small flat white colonies Small flat white colonies Small flat white colonies Small flat white colonies Small flat white color colonies	Small white colonies  Small flat white  Colonies  Small flat white  Colonies  Small flat white  Colonies  Small flat white  Colonies  Small flat white  Colonies  Small flat white  Colonies  Small flat white  Colonies  Small flat white  Colonies  Small flat white  Colonies  Small flat white  Colonies  Small flat white  Colonies  Small flat white  Colonies  Small flat white  Colonies  Small flat white color  Small flat white color  Colonies  Small flat white color  Slightly yellow color colonies	Small white colonies Manitolnon-fermenterpin pointed colonies Performed  Small flat white No change ,small white color colonies mucoid colonies Performed  Small flat white Yellow and pink colonies Not  colonies Performed  Small flat white Intense pink color colonies Not  colonies Performed  Small flat white yellow color colonies Not  colonies Performed  Small flat white Slightly pink color colonies Not  colonies Performed  Small flat white Slightly pink color colonies Not  colonies Performed  Small flat white color Fungal growth observe along with yellow color colonies colonies, No  hemolysis  Small flat white color Yellow ,flat, dry colonies Dark dull  colonies Dark dull  colonies Dark dull  colonies Dark dull  colonies Dark dull

**Table II.** Morphological characteristics of isolates from different grain samples.

Sample No.	Shape and Arrangement	Gram's Reaction	Position Of Spore
1.	Cocci in bunches & filamentous rods	Positive	No spores
2.	Cocci in bunches	Positive	No spores
3.	Thick Rods in chains	Positive	Central
4.	Coccobacillary	Positive	No Spores
5.	Cocci in bunches	Positive	No spores
6.	Scattered Rods	Positive	No Spores
7.	Cocci in bunches	Positive	No spores
8.	Cocci in bunches	Positive	No spores
9.	Cocci in tetrads and bunches & filamentous rods	Positive	No spores

**Table III.** Cultural characteristics of isolates from sterile swab samples from the walls, corners & backside of storage bags of different godowns & grocery shops of Pakistan.

Sample No.	Colonial Morphology on Nutrient Agar	Colonial Morphology on MacConkey's Agar
1.	Swarming growth	Yellow color colonies
2.	Swarming growth	No growth
3.	Small,flat white color colonies	Pink color, gummy colonies
4.	Small, flat white color colonies	Yellow color colonies
5.	Small,flat white color colonies	Pink color, gummy colonies
6.	Small, flat white color colonies	Pink color, gummy colonies
7.	Small,flat,yellow color colonies	Pink color, gummy colonies
8.	Small,flat white color colonies	Yellow color colonies, with black pigmentation
9.	Small,flat yellow color colonies	Yellow color, dull and gummy colonies
10.	Small,flat white color colonies	Yellow color, dull and gummy colonies

**Table IV.** Morphological characteristics of isolates from sterile swab sample from the walls, corners &backside of storage bags of different godowns & grocery shops of Pakistan.

Sample No.	Shape And Arrangement	Gram's Reaction	Position of Spore
1.	Cocci scattered & tetrads, coccobacillary	Positive	No spores
2.	Thick rods & coccobacllary	Positive	No spores
3.	Coccobacillary & rods	Positive	No spores
4.	Rods	Negative	Central
5.	Cocci &coccobacillary	Mix culture	No spores
6.	Short rods	Negative	No spores
7.	Cocci & rods in chains	Positve	No spores
8.	Thick rods in chains	Positive	Central
9.	Thick rods in chains	Positive	Central
10.	Thick rod in chains	Positve	Central

### **DISCUSSION**

In the present study, gram positive species i.e *Bacillus sp.*, *Staphlococcus sp.*, *Listeria sp.*, *Clostridium sp.* with few negative species. Mostly these splitted grains interact with air environment so these organism are continuously present in the air and get attach to these grains for growth thus these organism being gram

positive cannot cause serious disease as gram negative organism do, but still they are source of infection. Mainly these grains chewed by rodents had appear in night or at times when no person is there in the godowns ,so these rodents (rats, lizards, insects) can transfer their infections to the splitted grains. As gram negative bacteria are well known to spread infection in severe form and most of the fatal

diseases like brucellosis, plaque, lyme disease, listeriosis, *E.coli* infections, influenza the most common and many more zoonoses are spread via gram negative organisms.

In the present study, coagulase positive *Staphylococcus aureus* were isolated which is one of the most common and considered as potent pathogen, causes a wide range of suppurative infections, as well as food poisoning and toxic shock syndrome.

Gram positive endospores forming species isolated in this study are *Bacillus spp*. Aerial distribution of the dormant spores probably explains the occurrence of aerobic spore formers in most habitats examined.

Feacal matter organism isolation was also performed. Rats and mice mostly live in house sewerage pathways or animal farms from where they can pick *E. coli* by eating contaminated feed, drinking water, wastes through poultry and cattle excreta and also from infected herbage. *E. coli* in the present study remained the most prevalent bacteria isolated and identified form the feacal matter of rats, which is in agreement with Cizeket al. (1999), who reported that 5.3% of rodents stool samples were found positive for *E. coli* in Czech Republic.

From the present study it is concluded that the improper storage of grains and prevalence of rodents in go downs and grocery shops contribute a lot in the spread of zoonosis. Grains must be sealed properly and not store for longer period of time untreated from mites and ticks which develop and cause damage to grains. The shopkeeper must clean the dropped grains from packets during selling these dropped grains attract rodents and may be mixed with the non contaminated fresh grains and can contribute in the spread of zoonoses. The spread of zoonoses by rodents may be due to improper storage of grains in godowns, grocery stores, the rodents cause excessive spillage which

become contaminated with rodents excreata, dust, urine, saliva etc (Niethammer, 1981).

Proper grain storage, hygiene conditions, abundance of knowledge about zoonoses, proper facilities provided to diagnose and treat zoonotic infection, development and conservation of vaccine and prophylaxis, proper handling of animal and treating the infection carrier animals, improving environmental factors can contribute a lot in decrease of zoonoses and future of the world will be secure from these fatal infections.

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