Original Article

ASSESSMENT OF FOOD BORNE PATHOGENS IN DAIRY PRODUCTS

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ABSTRACT

Food is a basic necessity of life. It promotes growth and help in preventing and curing diseases. Ingestion of food containing pathogenic microorganisms causes food borne infections (FBI). Food borne illness caused by consuming bacterial contaminated poultry products are now growing public health concern. Present study was design to investigate the microbial load in egg contents and their susceptibility from antibiotics. For this 10 chicken egg samples were randomly collected from different areas in Karachi city. In this study of microbial assessment of egg contents the percentage of gram negative bacteria members of the family enterobacteriaceae is predominantly occurring is 80% which includes: E.coli (20%), Salmonella (30%), Proteus (10%), Klebsiella (10%) and Enterobacter (10%), while proportion of gram positive bacteria is comparatively low (20%) this includes Staphylococcus aureus (10%) and Bacillus spp. (10%). Salmonella is largely isolated species which 30% of all isolated bacteria because of their better ability to achieve invasion in reproductive tissue. After screening antibiotic sensitivity, all isolated strains shows effective zone of inhibition against Ofloxacin. The findings from this study therefore present a potential health problem to the populace. We recognize that eggs bear the bacterial contamination due to bad storage condition, high temperature, poor personal hygiene of food handlers, washing of egg with contaminated water and transport conditions. For this reason it is crucial to educate the public about good sanitary practices in handling eggs and preparing them for consumption which definitely play considerable role in minimizing bacterial load from eggs.

Introduction

Food is a chemically complex matrix (Easa, S.M.H., 2010) and it is a basic necessity of life, derives its importance from the fact that it supplies a variety of ingredients that give energy thus promoting growth and help in preventing and curing diseases (Irenkwe, G.E., 2012). Several factors contribute to, prevent, or limit the growth of microorganisms in foods; the most important are water availability, pH, and temperature. Due to the busy and hectic life schedule the traditional or conventional way of cooking is over and fast food industry is now emerging in most parts of the world. It was investigated that fast food lacking in proteins, vitamins and fiber thus facilitates the development of many microorganisms so the consumption of fast food in the world has been associated with many human diseases (Easa, S.M.H., 2010). Food borne infections (FBI) are growing public health problem with considerable economic and communal effects caused by the ingestion of food containing pathogenic microorganisms (Adebukola et al.2015). Food borne illness caused by consuming bacterial contaminated poultry products is a major cause of diarrheal diseases and effecting developed and under developed countries. Media generated reports about bacterial contaminations of poultry products can significantly affect public demand for these products and thus have correlated with negative impact on poultry industry and economic loss as well. (Abdullah, I.N., 2010). Egg is an excellent example of poultry product that's best, easy to use, convenient source of nutritious food for people (El-Kholoy et al.2014). An egg has high degree of natural defense mechanism against the contaminating microbes. Externally the three structures of egg are very much efficient in preventing the penetration of microorganisms; such as cuticle, hard calcium shell and waxy shell membrane (Msallam, A.K., 2008). Several egg white proteins present in albumin that bears antimicrobial properties especially the lysozyme. Another proteinase ovomucoid retard microbial ability to utilize albumin protein. Furthermore, viscosities of egg white and its pH which is about 9-10 (9.6) are not desirable for microbial growth. Natural defense mechanism of egg degrades over time that's why stored and aged eggs are at high risk to become infected as compare to fresh eggs (Chaemsanit et al. 2015). The shelf life of the eggs can be
prolonged in refrigerators because of their cold, dark and odorless environment. There are different opinions about whether the washing of eggs is beneficial or not. According to some researchers washing of egg result in decreased microbial counts on shells thus prevents the penetration of microorganisms inside the eggs through shells. On the other hand, some researchers claimed that washing can damage outer cuticle of the egg shell. This cuticle act as a barrier to contaminant's but after its removal there is an increase risk of deterioration of the internal quality of the egg (Nordenskjöld, J., 2010). Presence of moisture during temperature differential period immediately after lay; also contribute to bacterial penetration of egg shell (Webbet al. 2014). Microorganisms can contaminate the eggs at different stages, from production through processing to preparation and consumption (De Reu, Ket al. 2006). They can contaminate the egg at both egg shell and egg content with a wide range of pathogens such as members of enterobacteriaceae family, especially Salmonella. Egg shell contamination occurs after laying nesting material, dirt, and fecal matter. It was investigated that the egg shell flora is dominated by gram positive cocci because of their ability to tolerate dry and harsh conditions while the gram negative rods are also found in abundance (Adebukola et al. 2015). Liquid eggs are profusely dominated by gram negative types, such as members of enterobacteriaceae family while improper washing, storage methods and commercial egg breaking operations may contribute large numbers of gram positive cocci to the liquid egg. Eggs and egg products are positively cocci to the liquid egg. Eggs and egg products are predominantly contaminated with Salmonellaspp. (Easa, S.M.H., 2010). It is in general agreed that the microbial flora of hen’s egg at the time of laying is very low. The shell gets its load of microorganisms at oviposition, also from nesting material, trays, soil, dust and feces (Al-Ashmawy, M.A., 2013). Cracked eggs, dirty shells and unhygienic storage conditions are major reasons of egg contamination (Chaemsanit et al. 2015). There are two possible routes by which bacterial flora can contaminate the egg either vertically or horizontally (De Reu, Ket al. 2007). In vertical transmission, bacterial reproductive organs like ovaries and oviduct tissue can directly contaminate the yolk, the albumen or the membranes, before the eggs are covered by the shell (De Reu, K., 2006). Among different serotypes the prevalence of Salmonella enteritidis may be high in reproductive tissue with a wide range of pathogens including Salmonella. Egg shell contamination occurs after laying nesting material, dirt, and fecal matter. It was investigated that the egg shell flora is dominated by gram positive cocci because of their ability to tolerate dry and harsh conditions while the gram negative rods are also found in abundance (Adebukola et al. 2015). Liquid eggs are profusely dominated by gram negative types, such as members of enterobacteriaceae family while improper washing, storage methods and commercial egg breaking operations may contribute large numbers of gram positive cocci to the liquid egg. Eggs and egg products are predominantly contaminated with Salmonellaspp. (Easa, S.M.H., 2010). It is in general agreed that the microbial flora of hen’s egg at the time of laying is very low. The shell gets its load of microorganisms at oviposition, also from nesting material, trays, soil, dust and feces (Al-Ashmawy, M.A., 2013). Cracked eggs, dirty shells and unhygienic storage conditions are major reasons of egg contamination (Chaemsanit et al. 2015). There are two possible routes by which bacterial flora can contaminate the egg either vertically or horizontally (De Reu, Ket al. 2007). In vertical transmission, bacterial reproductive organs like ovaries and oviduct tissue can directly contaminate the yolk, the albumen or the membranes, before the eggs are covered by the shell (De Reu, K., 2006). Among different serotypes the prevalence of Salmonella enteritidis may be high in reproductive tissue because of their better ability to achieve invasion (Risk Assessment Studies, Report No.16.2004). On the other hand, horizontal transmission (trans-shell transmission) is usually derived from penetration of microorganisms through the eggshell. We often see visible fecal contamination on egg shell because during the laying process, egg passes through the highly contaminated cloaca area. Environmental vectors, such as farmers, pets and rodents also contribute to horizontal transmission (De Reu, Ket al. 2007).

**MATERIALS AND METHODS**

**SAMPLING:**

During the study period 10 chicken egg samples were randomly collected from different bakeries in Karachi city.

**SAMPLE PREPARATION:**

Each egg sample were dipped in 75% ethanol for 5 minutes then allowed it to air dry. The upper end of egg was famed for 5-10 minutes with Bunsen burner then hole was made with sterilized implement through which whole egg content was transferred into sterile beaker. The egg content was then blended for 30 seconds after which the mixture was used to inoculate appropriate bacteriological media.

**ISOLATION OF PATHOGENIC BACTERIA:**

Detection of Salmonella:

- 25 ml of egg content was transferred into 225 ml of buffer peptone water as pre enrichment.
- Incubated overnight at 37°C.
- 1 ml of the pre-inoculated culture was added into 9 ml of selenite cystine broth and incubated at 37°C for 24 hours for selective enrichment.
- A loopful from selenite cystine broth was then streaked onto Bismuth Sulphite Agar (BSA) and incubated for 24-48 hours at 37°C.
- Black coloured colonies were presumptively considered Salmonella.

Detection of Other Gram Negative Bacteria:

- 1 ml of egg content was inoculated into 9 ml lactose broth.
- Incubated for 24 hours at 37°C.
- Take a loopful from lactose broth and streaked on Macconkey Agar and Eosin Methylene Blue agar (EMB) and incubated for 24-48 hours.
- Colonies were differentiated on the basis of gram staining, morphology and biochemical tests.

Detection Of Gram Positive Bacteria:

- 25 ml of egg content was inoculated into 225 ml of buffer peptone water.
- Incubated at 37°C for 24 hours as pre enrichment.
- Loopful from pre enrichment streaked on nutrient agar plate and incubated overnight at 37°C.
- Colonies were initially differentiated on basis of morphology and gram staining.
- Colonies were sub cultured on Mannitol salt agar (MSA) and Blood agar.
- The isolates were further confirmed with the help of biochemical tests.

**BIOCHEMICAL CHARACTERIZATION OF MICROBIAL ISOLATES:**

The isolated colonies were pure cultured and then gram stained. Several biochemical tests were performed; slide coagulase, oxidase, catalase, IMVIC, triple sugar iron agar (TSI) test and urease test.

**ANTIBIOTIC SENSITIVITY TEST:**

Disc diffusion assay was performed to check antibiotic sensitivity of pathogenic bacteria by using broad spectrum antibiotic discs, Streptomycin (S) 10, Ofloxacine (OFX) 5, Oxacilllin (OX) 1, Tobramycin (TN) 10 and Penicillin (P) 1. Each strain culture was diluted with saline and match with 0.5 McFarland then with the help of swab makes a lawn over separate Mueller-Hinton Agar (MHA). Allow the surface to dry then by using sterile forceps place discs on the plate and incubated at 37°C for 16-24 hours. Zone of inhibition measured by using broad spectrum antibiotic discs, Streptomycin (S) 10, Ofloxacine (OFX) 5, Oxacilllin (OX) 1, Tobramycin (TN) 10 and Penicillin (P) 1. Each strain culture was diluted with saline and match with 0.5 McFarland then with the help of swab makes a lawn over separate Mueller-Hinton Agar (MHA). Allow the surface to dry then by using sterile forceps place discs on the plate and incubated at 37°C for 16-24 hours. Zone of inhibition measured...
in millimeter and were interpreted as sensitive, intermediate and resistant as per the Clinical Laboratory Standard Institute (CLSI) guidelines.

**RESULTS**

The results show a wide range of bacteria determined by direct microscopic examination and confirmation tests on selective media and conventional biochemical tests. All studied samples (10 samples) have shown heavy contamination of pathogens. These include 2 species of gram positive bacteria that is Staphylococcus aureus = 10%, Bacillus spp. = 10% and 5 species of gram negative bacteria that is Salmonella spp. = 30%, Klebsiella pneumoniae = 10%, Proteus spp. = 10%, E. coli = 20%, Enterobacter spp. = 10% (Table 1).

To determine antibacterial sensitivity of isolated bacterial strains 5 commercially available broad spectrum antibiotics are used which are Penicillin, Oxacillin, Ofloxacin, Tobramycin, and Streptomycin. Results are presented in Table 2 which point toward sensitivity, intermediary, and resistivity of isolated strains against antibiotics. All isolated strains show effective zone of inhibition against Ofloxacin.

**TABLE: 1 PREVALENCE OF BACTERIAL ISOLATES FROM EGG CONTENTS**

<table>
<thead>
<tr>
<th>BACTERIAL ISOLATES</th>
<th>PERCENTAGE</th>
</tr>
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<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>(10%)</td>
</tr>
<tr>
<td>Bacillus spp.</td>
<td>(10%)</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>(10%)</td>
</tr>
<tr>
<td>Enterobacter spp.</td>
<td>(10%)</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>(30%)</td>
</tr>
<tr>
<td>E. coli</td>
<td>(20%)</td>
</tr>
<tr>
<td>Proteus spp.</td>
<td>(10%)</td>
</tr>
</tbody>
</table>

**TABLE: 2 ANTIBIOTIC SUSCEPTIBILITY TESTING OF BACTERIAL ISOLATES**

<table>
<thead>
<tr>
<th>ANTIBIOTICS</th>
<th>BACTERIAL ISOLATES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E. coli</td>
</tr>
<tr>
<td>Streptomycin (S)</td>
<td>10mm</td>
</tr>
<tr>
<td>Oxacillin (Ox)</td>
<td>10mm</td>
</tr>
<tr>
<td>Ofloxacin (Of)</td>
<td>10mm</td>
</tr>
<tr>
<td>Tobramycin (To)</td>
<td>10mm</td>
</tr>
<tr>
<td>Penicillin (P)</td>
<td>10mm</td>
</tr>
</tbody>
</table>


Bar Diagram Showing antibiotic Susceptibility of Bacterial Isolates from Streptomycin, Ofloxacin, Oxacillin, Tobramycin, and Penicillin.

**DISCUSSION**

In this study, both gram positive and gram negative bacteria isolate from egg content this is correlated with previous study which found that rotten eggs normally contain a mixed infection of gram negative and a few gram positive organisms (Stadelman et al. 1995) although the percentage of gram negative bacteria members of the family Enterobacteriaceae that is usually predominantly occurring is 80% which includes E. coli, Salmonella, Proteus, Klebsiella, and Enterobacter. While proportion of gram positive bacteria is comparatively low (20%) this includes Staphylococcus aureus and Bacillus spp. This is in agreement with earlier study which reported microbial contamination of chicken eggs with predominantly gram negative bacteria members of the family Enterobacteriaceae (Salihuet al. 2015).

In current study, among gram negative bacteria Salmonella is largely isolated species which is 30% of all isolated bacteria. This result is also concurrence with the study of (Risk Assessment Studies Report No.16. 2004) in which highest percentage 36% Salmonella isolated from egg and egg products. Raw egg shell and its contents are well known to be a source of Salmonella due to their extraordinary ability to colonize ovarian tissues of hens (Salihuet al. 2015). Other species of gram negative bacteria which were isolated from egg content in this study is Klebsiella (10%), Proteus (10%), Enterobacter (10%) and E.coli (20%) these results also associated with previous study but percentages
of isolated organisms in pervious study is higher because of large sample size. Staphylococcus aureus and Bacillus are gram positive isolates of egg content of this study however; gram positive organisms predominantly present on egg shells so it might possible that they enter liquid eggs upon breaking. Earlier studies also reported isolation of Staphylococcus aureus and Bacillus from egg contents (Saharinaethet al. 2009).

In antibiotic sensitivity, Salmonella show sensitivity from Olofoxacin and Tobramycin this is also in agreement with previous study in which species of Salmonella show sensitivity from these antibiotics (Shah, A.H. and Korejo, N.A., 2012). In current study E. coli, Enterobacter and Klebsiella show sensitivity from Olofoxacin our results also correlate with previous study in which these bacteria show good sensitivity from Olofoxacin (Ezekiel, C.N et al. 2011). In present study Saureus show sensitivity from all assessed antibiotics however confer intermediate resistance from Pencillin but in earlier study Saureus become totally resistant from Pencillin (Fyzik et al. 2014). In current study Proteusshow susceptibility from Olofoxacin and Tobramycin which is also associated with preceding study (Dadheechet al. 2015).

CONCLUSION

In this study of microbial assessment of egg contents; we found that egg contents predominantly contaminated with enterobacteriaceae family, especially Salmonella however, gram positive isolates also there. We concluded that eggs bare the bacterial contamination due to bad storage conditions in storehouse, high temperature, bad sanitary condition, hand touching, and all other surrounding pollution state. Furthermore, cross-contamination of freshly laid sterile eggs by contaminated poultry feeds and washing of eggs with contaminated water may be a factor in increase eggs contamination. For this reason it is crucial to educate the public about good sanitary practices in handling eggs and preparing them for consumption, also consumers should keep eggs in refrigerator and cooked eggs well to kill bacteria. We strongly suggested that at the government should set quality control standards in the storage conditions of market eggs to minimize the potential risk of illness due to the consumption of egg and egg products.

REFERENCES