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# *Studies on the Prevalence of Enterobacteriaceae in Chickens and Chicken eggs*

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**This study was done to investigate the prevalence of the Enterobacteriaceae in chickens and eggs. Isolation of forty four different bacterial isolates belonging to Enterobacteriaceae from chicken egg samples, cloacal swabs and swabs from Hatcheries's floor, the isolates from commercial flock swabs were biochemically identified as E coli, P. mirabilis E Sakazakii and E .cloacae by incidence 22%, 55 %, 11% and 11 % respectively. The isolates from Layers and broilers breeder cloacal swabs were biochemically identified to be E. coli, P. mirabilis E. fergusonii and E .cloacae by incidence 20 %, 20 %, 20% and 40 % respectively. The isolates from commercial eggs were biochemically identified to be Pantoea Sp. , Kluyvera sp., E Sakazakii , E.aerogenes and E.harmanii by incidence 33.3% , 16.6% , 16.6% , 16.6% and 16.6 % respectively. The isolates from fertilized egg samples were biochemically identified as E Sakazakii , E. fergusonii , E.coli , E. Cloacae , Aeromonas ,S. Anatum and Prov. Alcolifaciens with a number of 1 ,1, 3, 3, 2, 2 and 1 , incidence 8% , 8% , 23% , 23% , 15% , 15% and 8 % respectively. The incidence of Enterobacteriaceae isolates from floor swabs of both primitive and automatic hatcheries was 20.8 % and these isolates were biochemically identified to be Pantoea spp., Citrokoserilama, k.pneumo. Ozaenae and E .cloacae with number 2, 1, 1 and 1 also its incidence were 40%, 20%, 20% and 20 % respectively. We found that the most common isolated bacterium from eggs either fertilized or commercial table eggs in our study was E.coli although we could isolate other bacterial species as Enterobacter, Proteus species , Escherichia fergusonii; E. Sakazakii, Klebsiella sp., S. anatum, and Pseudomonas sp..In-vitro sensitivity test of the isolated strains to various chemotherapeutic agents revealed that all isolates were sensitive to Ciprofloxacin, Enrofloxacin, and Amoxicillin.**

Enterobacteriaceae is a family of rod-shaped, aerobic, facultative anaerobic bacteria. The Enterobacteriaceae family is subdivided into 8 tribes including: Escherichieae, Edwardsielleae, Salmonelleae, Citrobactereae, Klebsielleae, Proteeae, Yersineae, and Erwineae. Ardrey *et al.,* (1968) isolated E coli from droppings contaminated eggs of layers at the level of 2.7% Labaque *et al.,* (2003) reported that the microbial contamination was higher (24%) in very dirty eggs than in eggs which were clean or dirty (16%).Collecting eggs soon after laying will reduce the risk of microbial contamination. Cecilia Rosario Cortés *et al.,* (2004) reported that E .coli were the most common bacterium that recovered from all samples except the sawdust and fertile eggs collected from the nest. Fertile egg contamination at breeder farm level was found to be minimal. Musgrove *et al.,* (2004) demonstrated that commercial processing decreased microbial contamination of eggshells. Also, prevalence of Enterobacteriaceae differs according to type of egg unwashed and commercially washed eggs. Proteus sp., Enterobacter sp., Pseudomonas sp., Klebsiella sp., Staphylococcus sp., Streptococcus sp., Clostridium sp., Bacillus cereus, S. typhimurium and Enterococcus have been isolated from hatching eggs. However, the most common isolated bacterium is E. coli. Shalaby and Abd El-Hamid (1987) reported that the isolated E. coli in prevalence of 44.5% from hatching eggs which responsible for embryonic mortalities in hatcheries. Ana *et al.,* (2001) found that the prevalence of Salmonella in chicken products and hen's eggs were 13 (10, 48%) Among the 13 strains of salmonellae isolated, 10 were serotyped as S. Enteritidis, 1 was S. Anatum and 2 were S. Enterica. Adesiyun *et al.,* (2005) reported the microbial quality of table eggs sold in Trinidad was conducted eggs (shells, egg content or both) sampled were positive for Salmonella, E. coli, and Campylobacter; respectively. Israa and Majeed (2011) conducted to detect E.coli in hatching eggs and premises in poultry hatcheries, Results revealed isolation of E.coli, Klebsiella sp. Proteus sp. and pseudomonas spp. Ramnoff, (1960) Fertilized

eggs contaminated with micro-organisms may result in weak chicks, poor chick growth and low feed conversion rate Jones *et al.,* (2011) studied the prevalence of coliforms, Salmonella, Listeria and Campylobacter associated with eggs and the environment of conventional cage and free range egg production flocks of laying hens.

## Material and methods

**Samples.** 377 samples were collected from Elminya and Beni-Suef governorates, samples were collected between August 2010 up to March 2012, samples were obtained from table eggs from a large-scale poultry farm (Cage system), a small-scale poultry farm (Deep litter system) and fertile egg samples from commercial hatcheries, Automatic incubator and from fertile egg producing farms. Fecal swabs from layers also collected and swabs from incubator floor, arranged as follow.

| Governorates | Type of sample | | | | Total No. of samples |
|---|---|---|---|---|---|
| | Fertile eggs | Table eggs | Cloacal swabs | Hatcheries swabs | |
| Al-Minia | 103 | 128 | 42 | 20 | 293 |
| Beni Suef | 10 | 50 | 20 | 4 | 84 |
| **Total** | 113 | 178 | 62 | 24 | 377 |

**Media.**

**Fluid and solid media for isolation.**

- **Selenit-F broth (SF)** (*Oxoid co.*). Used as a selective enrichement media for genus Salmonella
- **Tryptone Soya broth** (*Oxoid co.*). Used for cultivation of E. coli, P. mirabilis, and Enterobacter.
- **MacConkey bile salt lactose agar medium** (*Oxoid co.*). Used as differential medium for isolation of members Enterobacteriaceae and other Gram-negative bacteria with inhibition of Gram-positive micrococci
- **Salmonella Shigella agar** (*Oxoid co.*). Differential selective medium used for the isolation of members of Enterobacteriaceae. It differentiates between lactose and non-lactose fermenting organisms.
- **Xylose lysine decarboxylase agar (XLD)** (*Oxoid co.*). Differential selective medium used for the isolation of members of Enterobacteriaceae, and for identification of salmonellae.
- **Semi-solid agar** (*Oxoid co.*). Used as 0.3 % agar dissolved in nutrient broth for preservation of isolates and detection of the bacterial motility.
- **Nutrient agar medium.** Used for cultivation of isolated bacteria.

**Growth, biochemical characterization and Motility.**

- Growth on MacConkey agar media, Motility (on semi solid agar).
- Biochemical tests reagents. Oxidase test and API system (micro-method API 20E plate system-Biomerieux –France cat# 20-100) for the biochemical characterization of Enterobacteriaceae isolates

**Biological Materials.**

- 5% citrated sheep blood: It was used for preparation of Blood agar for detection of haemolytic characters.
- Chicken, Mice, Human, Camel, Cow, Buffalo, Horse and Sheep Red blood cells: It was used for haemagglutination test.

**Antibiogram assay.**

- **Mueller Hinton agar** (*Oxoid co.*). It produces large-clear zones of inhibition when sensitive organisms meet susceptible antibiotic by using the disc diffusion method.
- **Antimicrobial discs** (*Oxoid co.*). The following antibiotic discs were used: Amoxicillin (Ax 25 µg), Enrofloxacin (ENR 5 µg), Ciprofloxacin (CIP 5 µg), Deoxycyclin (Do 30 µg), Neomycin (N 30 µg) and cephadrine (CE 3 µg).

Forty four Enterobacteriaceae organisms isolated from chicken eggs were thoroughly characterized by standard cultural and biochemical tests. From which 24 were tested for susceptibility to 6 antibiotics following disk diffusion method (Akond *et al.,* 2009).

**The disc diffusion technique.** This technique was conducted according to Finegold and Martin (1982). Adjusted to Mcfarland's opacity tube no. 0.5 (corresponding to $1.5x\ 10^8$ cfu/ml). These results were interpreted according to the manufacturing company as shown in Interpretation results as shown in (Table 9)

**Bacteriological examination**

**Isolation of bacterial agents.**

- Cloacal swabs. Samples were taken according to Papadopoulou *et al.,* (1997) and Cheesbrough (2000). Cloacal cotton swabs were immersed into 10 ml Selenit F. broth and also 10 ml

Tryptone Soya agar, incubated aerobically at 37˚C for 18 hours.

- Eggs samples. Swabbing of egg surfaces by Selenit F broth according to Moustafa-Sabah, (1993); Stępień-Pyśniak, (2010)
- Swabs from hatcheries `s floor. Swabbing of primitive and automatic hatcheries surfaces by cotton swabs immersed in selenit F broth. Four swabs were taken from each side of each hatchery.

**Identification of the isolates.** The pure colonies of the isolates were identified according to Collier *et al.,* (1998).

**Colonial Morphology.** Pure culture colonies from each isolate were identified morphologically according to Cruichshank *et al.,* (1975); Quinn *et al.,* (2002).

**Gram's staining.** Stain character of the obtained bacterial isolates was carried out according to Collee *et al.,* (1989).

**Biochemical identification.** Api20E: - Biochemical identification of the isolated bacterial colonies was performed using the API 20E test (*Biomerieux, France*).

API 20 E is a standardized identification system for Enterobacteriaceae and other non-fastidious, Gram-negative rods which use 21 miniaturized biochemical tests and a database.

**Detection of virulence factors.**

- Detection of virulence factors of E. coli isolates: A total of five E. coli strains were tested for detection and evaluation of their virulence factors.
- Detection of haemolytic activity: Blood agar base containing 5% citrated sheep blood was streaked with overnight cultures of isolated strains of E. coli and incubated at 37˚C for 24 hr. Complete haemolysis was recognized as β-haemolysis while, weak incomplete haemolysis was recognized as haemolysis (Marilda *et al.,* 1990).
- Detection of haemagglutination and mannose resistance of haemagglutination using Human, Sheep, Camel, Horse, Cow, Buffalo, Mice and Chicken RBCs according to Marilda *et al.,* (1990).

## Results and discussion

In our study, 44 different bacterial isolates belonging to Enterobacteriaceae, including E coli, E. cloacae, E. fergusonii, E. Sakazakii, E.aerogenes, E.harmanii, Enterobacter, Klebsiella, Proteus mirabilis, Pseudomonas aeruginosa, Pantoea sp., Kluyvera peumOzaenae, Citro.Koseri, Salmonella Anatum, Aeromonas and Prov. Alcolifaciens, could be isolated from chicken egg samples either commercial table eggs or from fertilized eggs, Cloacal swabs and swabs from Hatcheries's floor and these results agreed with Ardrey *et al.,* (1968) as stated that the members of Enterobacteriaceae like E coli isolated from droppings laid contaminated eggs of layers at the level of 2.7%. That is explaining our results (Table 2) as we isolated E.coli (20%), E. Cloacae (40%), E. fergusonii (20%) and P. mirabilis (20%) from fertilized eggs and cloacal swabs from hens which laid these fertilized eggs; also we were isolate P. mirabilis (55.5%), E.coli (22%), E.Sakazakii (11%) and E.Cloacae (11%) from commercial table eggs immediately after being laid and from cloacal swabs from hens which laid these commercial table eggs and these results were agreed with Berrang *et al.,* (1999) as stated that adhered organisms on the eggshell surface to broken eggshell may infect the egg components. And with (Yousseif and Geissler, 1985) which state that the Proteus mirabilis were isolated from non fertile eggs, dead in shell embryos and dead chicks. The Forty four different bacterial isolates of Enterobacteriaceae these isolates were grown on MacConkey, Salmonella Shigella, and nutrient and XLD agar (Table 1).

On MacConkey agar, lactose fermenting bacteria utilized the lactose available in the medium and results in the appearance of red/pink colonies and the medium surrounding the colony was opaque such as E coli, Enterobacter and Klebsiella while Non lactose fermenting bacteria cannot utilize lactose resulting in formation of white/colorless colonies formed in the plate. They can also look golden to brown with dark centers such as Proteus species and Escherichia fergusonii; they can also formed light to dark pink colonies as in E. Sakazakii strains. On SS agar colonies of non-lactose fermenting appeared transparent with black centers while colonies of lactose fermenting were pink. In the same time on XLD agar colonies of non-lactose fermenting appeared black with colorless margins indicating $H_2S$ production while other members non $H_2S$ production were yellow colonies with or without coloration of media.

We found that E.coli was the most common isolated bacterium from eggs either fertilized or commercial table eggs Table(3,4,5 and 6) and also we could isolated other bacterial species as Enterobacter , Klebsiella Proteus species , Escherichia fergusonii; E. Sakazakii, Klebsiella sp., S. anatum, and Pseudomonas sp. and these

results were agreed with (Sarma *et al.,* 1985 and Cason *et al.,* 1994) as they have been isolate Proteus sp., Enterobacter sp., Pseudomonas sp., Klebsiella sp., Staphylococcus sp., Streptococcus sp., Clostridium sp., Bacillus cereus, S. typhimurium and Enterococcus from hatching eggs. However, the most common isolated bacterium is E.coli.

We isolated E. fergusonii by (20%) from fertilized eggs and cloacal swabs from hens which laid these fertilized eggs these results Table (3, 6) agreed with the results of Herraez *et al.,* (2005) who reported that the E. fergusonii was a member of Enterobacteriaceae, closely related to E.coli and Shigella sp., established as a new species of the genus Escherichia in (1985). These bacteria are potential pathogens as new strains arise all the time from natural mutations including particularly virulent strains.

E. coli isolates were characterized by their ability to utilize the following sugars: maltose, lactose, sucrose, dulcitol, adonitol, salicin, raffinose, dextrin, xylose, rhamnose and mannitol while negative reaction with ODC, CIT, H2S , URE, TDA, IND, VP, GEL, SAC, AMY and OX tests. E. Sakazakii is also distinguished by its ability to ferment sucrose, raffinose, and a-methyl-D-glucoside, but not D-sorbitol, dulcitol, adonitol, or D-arabinol . E. cloacae isolates were detected by their ability to utilize the following sugars: maltose, lactose, sucrose, dulcitol, adonitol, salicin, raffinose, dextrin, xylose, rhamnose and mannitol While negative reaction with LDC, GEL , INO, H2S , URE, TDA, IND and OX. P.mirabilis only fermented glucose while negative reaction with ONPG, CIT, LDC, SAC, MAN, AMY, SOR, RHA, MEL, IND, ARA and OX tests.

The most effective antibiotic therapy against Enterobacteriaceae infection we founded that the most sensitive antibiotic and effective one was ciprofloxacin as when used for treatment of experimentally infected groups lead to decrease clinical sings and improvement of performance parameters and the histopathological picture for intestine show mild degenerative change in treated groups in comparison with the non ciprofloxacin treated groups that agreed with Gowda *et al.,* (1997) reported that most of the 105 E coli isolates which were recovered from poultry with colisepticaemia were sensitive to flumequine, norofloxacin, ciprofloxacin and pefloxacin. They were highly resistant to amoxicillin, ampicillin and erythromycin and with Arathy *et al.,* (2011) determined that the antimicrobial resistance profile of E coli isolated from the shell membrane and yolk of commercial chicken eggs in Grenada was observed the highest for Ampicillin and The lowest resistance rate among all the antibiotics was observed against Enrofloxacin. Israa and Majeed (2011) stated that E coli isolated from hatching eggs and premises in poultry hatcheries was highly sensitive to Imipenem , Amikacin , Cefotaxin, meanwhile it was less sensitive to Norofloxacin, Ciprofloxacin, but it was resistant to Gentamycin, Cephaloxin, Ampicillin and Trimethoprime. And the withdrawal period of this ciprofloxacin is short period so there is no residual effect or public hazard these results was seemed to matched with the results of (Herraez *et al.,* 2005) the effective use of antibiotic therapy results usually in the eradication of the infecting organism and with (Hamdy, *et al.,* 1983) which said that Poultry bacterial drug resistance pathogens represent a potential health hazard for human as they leave a drug residue in poultry products but this is disagreed with Butura *et al.,* (1973) which found that 98% of E. coli isolates recovered from diseased poultry was sensitive to Chloramphenicol, 97% to Polymyxin B, 81% to Furazolidone, 78% to Neomycin, 32% to Tetracycline, but all were resistant to Erythromycin and Streptomycin and (Rao *et al.,* 1976) Isolated 347 strains of E coli from poultry. They found that the highest incidence of drug resistance was encountered mainly to erythromycin (98.8%), Streptomycin (93.3%), oxytetracycline (86.6%) and chlortetracycline (76%). No strains were resistant to ampicillin and chloramphenicol. Resistance to nitrofurazone was found in 2.9% of the strains.

## Conclusions

The conclusion of our results are the strict hygienic measures and good sanitation of hatcheries and eggs prevent the egg contamination by Enterobacteriaceae infection and not use the incubators for incubating different types of poultry eggs as in our work we isolate Salmonellae Anatum which is predominately present in ducks and that explain the contamination of eggs from incubator without good sanitation, also the using of the most effective antibiotics for treatment of infected hens to prevent the transfer of bacteria in the oviduct

Using of ciprofloxacin has a great effect against Enterobacteriaceae infection.

**Table (1):** Features and number of isolated Enterobacteriaceae on MacConkey agar.

| | Lactose fermenter | Non lactose fermenter |
|---|---|---|
| **Colony appearance** | Red/pink colonies | White/colorless or golden to brown with dark centers colonies |
| **Media color** | Hazy (opaque) | Lighter in color |
| **Bacteria** | E coli,<br>E. cloacae<br>E. Sakazakii<br>Klebsiella | Proteus species<br>Pseudomonas aeruginosa<br>E. fergusonii |
| **No** | 25 | 19 |

**Table (2):** Identification of bacterial isolated from cloacal swabs collected from table egg producing farms.

| Flock no. | Swabs no. | Isolates | % | *E. coli* | | *P. mirabilis* | | *E. Sakazakii* | | *E. cloacae* | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | No | % | No | % | No | % | No | % |
| 1 | 5 | - | - | - | - | - | - | 1 | 5 | - | - |
| 2 | 5 | 1 | 20 | - | - | 1 | 11 | 2 | 5 | 1 | 20 |
| 3 | 4 | 1 | 25 | - | - | - | - | 3 | 4 | 1 | 25 |
| 4 | 5 | 2 | 40 | - | - | 1 | 11 | 4 | 5 | 2 | 40 |
| 5 | 5 | 1 | 20 | - | - | 1 | 11 | 5 | 5 | 1 | 20 |
| 6 | 5 | 2 | 40 | 1 | 11 | 1 | 11 | 6 | 5 | 2 | 40 |
| 7 | 5 | 1 | 20 | 1 | 11 | - | - | 7 | 5 | 1 | 20 |
| 8 | 5 | 1 | 20 | - | - | 1 | 11 | 8 | 5 | 1 | 20 |
| **Total** | **39** | **9** | **23** | **2** | **22** | **5** | **55.5** | **Total** | **39** | **9** | **23** |

**Table (3):** Identification of bacteria isolated from Cloacal swabs collected from fertilized egg producing farms.

| Flock no | Swabs no | Isolates | % | *E. coli* | | *P. mirabilis* | | *E. fergusonii* | | *E. cloacae* | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | No | % | No | % | No | % | No | % |
| 1 | 5 | 1 | 20 | 1 | 20 | - | - | - | - | - | - |
| 2 | 4 | 1 | 25 | - | - | - | - | - | - | 1 | 25 |
| 3 | 5 | 1 | 20 | - | - | - | - | 1 | 20 | - | - |
| 4 | 5 | 1 | 20 | - | - | 1 | 20 | - | - | - | - |
| 5 | 4 | 1 | 25 | - | - | - | - | - | - | 1 | 25 |
| **Total** | **23** | **5** | **22** | **1** | **20** | **1** | **20** | **1** | **20** | **2** | **40** |

**Table (4):** Identification of bacterial isolates obtained from commercial eggs.

| Farm | No | Isolates | % | *Pantoea Spp* | | *Kluyvera spp.* | | *E. Sakazakii* | | *E. aerogenes* | | *E. harmanii* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | No | % | No | % | No | % | No | % | No | % |
| 1 | 10 | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 15 | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 15 | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 15 | 2 | 13.33 | - | - | - | - | 1 | 16.66 | 1 | 16.66 | - | - |
| 5 | 15 | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 15 | - | - | - | - | - | - | - | - | - | - | - | - |
| 7 | 18 | 1 | 5.55 | 1 | 16.66 | - | - | - | - | - | - | - | - |
| 8 | 15 | - | - | - | - | - | - | - | - | - | - | - | - |
| 9 | 15 | 1 | 6.66 | - | - | 1 | 16.66 | - | - | - | - | - | - |
| 10 | 15 | - | - | - | - | - | - | - | - | - | - | - | - |
| 11 | 15 | 1 | 6.66 | - | - | - | - | - | - | - | - | 1 | 16.66 |
| 12 | 15 | 1 | 6 | 1 | 16.66 | - | - | - | - | - | - | - | - |
| **Total** | **178** | **6** | **3.37** | **2** | **33.3** | **1** | **16.66** | **1** | **16.66** | **1** | **16.66** | **1** | **16.66** |

**Table (5)**: Identification of Bacterial isolates obtained from fertilized eggs,* fertilized eggs from primitive hatchery, ** fertilized eggs from automatic hatchery.

| Farm no | No of Eggs | No of Isolates | % | *E. Sakazakii* | | *E. Fergusonii* | | *E coli* | | *E. Cloacae* | | *Aeromonas* | | *S. Anatum* | | *Prov. Alcolifaciens* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | No | % | No | % | No | % | No | % | No | % | No | % | No | % |
| 1 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 10 | 1 | 10 | 1 | 7.69 | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 10 | 1 | 10 | - | - | 1 | 8 | - | - | - | - | - | - | - | - | - | - |
| 5 | 13 | 2 | 15.38 | - | - | - | - | 1 | 8 | 1 | 8 | - | - | - | - | - | - |
| 6* | 10 | 2 | 20 | - | - | - | - | - | - | - | - | - | - | 2 | 15 | - | - |
| 7* | 6 | 2 | 33.33 | - | - | - | - | 1 | 8 | 1 | 8 | - | - | - | - | - | - |
| 8* | 5 | 2 | 40 | - | - | - | - | - | - | - | - | 1 | 8 | - | - | 1 | 8 |
| 9* | 10 | 1 | 10 | - | - | - | - | - | - | 1 | 8 | - | - | - | - | - | - |
| 10* | 9 | 1 | 11.11 | - | - | - | - | 1 | 8 | - | - | - | - | - | - | - | - |
| 11* | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 12** | 5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 13** | 5 | 1 | 20 | - | - | - | - | - | - | - | - | 1 | 8 | - | - | - | - |
| **Total** | **113** | **13** | **11.5** | **1** | **7.69** | **1** | **7.69** | **3** | **23** | **3** | **23** | **2** | **15** | **2** | **15** | **1** | **8** |

**Table (6):** Over all data about microbiological detection of Enterobacteriaceae in Eggs (291) from 25 farms.

| Item | Isolates | *E.coli* | *Aeromonas* | *E. Cloacae* | *S. Afula* | *E. fergusonii* | *Prov. Alcolifaciens* | *Pantoea Spp.* | *Kluyvera Spp.* | *E. Sakazakii* | *E.aerogenes* | *E.harmanii* | *S. anatum* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No | 19 | 3 | 2 | 3 | 1 | 1 | 1 | 2 | 1 | 2 | 1 | 1 | 1 |
| % | 6.5 | 15.78 | 10.5 | 15.78 | 5.26 | 5.26 | 5.26 | 10.5 | 5.26 | 10.5 | 5.26 | 5.26 | 5.26 |

**Table (7):** Identification of bacterial isolates obtained from Hatcheries floor swabs.

| Hatchery No | Number of Swabs | Number of Isolates | % | *Pantoea spp.* | | *Citrokoseri lama* | | *k. pneumozaenae* | | *E. cloacae* | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | No | % | No | % | No | % | No | % |
| 1* | 4 | 1 | 25 | 1 | 20 | - | - | - | - | - | - |
| 2* | 3 | 1 | 33 | - | - | 1 | 20 | - | - | - | - |
| 3* | 3 | - | - | - | - | - | - | - | - | - | - |
| 4* | 3 | - | - | - | - | - | - | - | - | - | - |
| 5* | 4 | 1 | 25 | - | - | - | - | 1 | 20 | - | - |
| 6* | 3 | 1 | 33 | 1 | 20 | - | - | - | - | - | - |
| 7** | 2 | 1 | 50 | - | - | - | - | - | - | 1 | 20 |
| 8** | 2 | - | - | - | - | - | - | - | - | - | - |
| **Total** | **24** | **5** | **20.8** | **2** | **40** | **1** | **20** | **1** | **20** | **1** | **20** |

* Primitive hatchery ** Automatic hatchery

**Table (8):** All biochemical reactions of all bacterial isolates.

| Bacterial spp. | API 20 E RESULTS | | | | | | | | | | | | | | | | | | | | | Recovery rate (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ONPG | ADH | LDC | ODC | CIT | H2S | URE | TDA | IND | VP | GEL | GLU | MAN | INO | SOR | RHA | SAC | MEL | AMY | ARA | OX | |
| *E. coli* | + | + | + | - | - | - | - | - | + | - | - | + | + | - | + | + | - | + | - | + | - | **99** |
| | + | + | + | - | - | - | - | - | + | - | - | + | + | - | + | + | - | + | - | + | - | **98** |
| | + | - | + | - | - | - | - | - | + | - | - | + | + | - | + | + | - | + | - | + | - | **99** |
| *E. fergusonii* | - | - | + | + | - | - | - | - | + | - | - | + | + | - | - | + | - | - | + | + | - | **99.5** |
| | - | - | + | + | - | - | - | - | + | - | - | + | + | - | - | + | - | - | + | + | - | **99.5** |
| *P. mirabilis* | - | - | - | + | - | + | + | + | - | + | + | + | - | - | - | - | - | - | - | + | - | **99.9** |
| *E . Sakazakii* | + | + | - | + | + | - | - | - | - | + | + | + | + | + | - | + | + | + | + | + | - | **99** |
| | + | + | - | + | + | - | - | - | - | + | + | + | + | + | - | + | + | + | + | + | - | **98** |
| | + | + | - | + | + | - | - | - | - | + | + | + | + | + | - | + | + | + | + | + | - | **99** |
| *E . Cloacae* | + | + | - | + | + | - | - | - | - | + | - | + | + | - | + | + | + | + | + | + | - | **95.2** |
| | - | + | + | + | + | - | + | - | - | + | - | + | + | + | + | + | + | + | + | + | - | **70** |
| | - | + | + | + | + | - | + | - | - | + | - | + | + | + | + | + | + | + | + | + | - | **95** |
| | - | + | + | + | + | - | + | - | - | + | - | + | + | + | + | + | + | + | + | + | - | **99.9** |
| *Pantoea spp* | - | - | - | - | + | - | - | - | - | - | - | + | + | - | - | + | + | + | + | + | - | **95** |
| | - | - | - | - | + | - | - | - | - | - | - | + | + | - | - | + | + | + | + | + | - | **91.3** |
| *Kluyvera spp* | - | - | - | + | + | - | - | - | + | - | - | + | + | - | + | + | + | + | + | + | - | **60** |
| *K . pneumozaenae* | + | + | - | - | - | - | - | - | - | - | - | + | + | + | - | + | - | + | + | + | - | **77** |
| *Citro.Koseri* | - | + | - | + | + | - | - | - | + | - | - | + | + | - | + | + | - | - | + | + | - | **98.7** |

**Table (9):** Results of sensitivity test for isolates, Conc: concentration of antimicrobial agent.

| Antimicrobial agents | Code | Conc (µg) | Susceptible zone diameter | | |
|---|---|---|---|---|---|
| | | | Resistant | Intermediate | Sensitive |
| **Amoxicillin** | AML | 10 | 13 | 14-17 | 18 |
| **Ciprofloxacin** | CIP | 5 | 13 | 14-16 | 17 |
| **Neomycin** | N | 30 | 12 | 13-16 | 17 |
| **Deoxycyclin hydrochloride** | DO | 30 | 12 | 13-15 | 16 |
| **Enrofloxacin** | ENR | 5 | 12 | 13-16 | 17 |
| **Clindamycin** | C | 2 | 12 | 12-14 | 14 |

**Table (10):** Results of sensitivity test for bacterial isolates.

| Antibiotics | Conc (µg) | *E.coli* | *E.fergusonii* | *E.sakazakii* | *P.mirabilis* |
|---|---|---|---|---|---|
| **Amoxicillin** | 25 | R | R | R | R |
| **Ciprofloxacin** | 5 | S | S | R | S |
| **Cephadrine** | 3 | R | R | R | R |
| **Neomycin** | 30 | S | S | S | IS |
| **Deoxycyclin** | 30 | R | R | R | R |
| **Enrofloxacin** | 5 | R | R | R | R |
| **Clindamycin** | 2 | R | R | R | R |

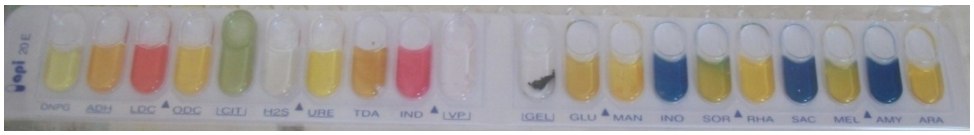


**Photo (A) of E. coli:** Showing positive reaction with ONPG, LDC, ADH, , GLU, MAN, INO, SOR, RHA, MEL and ARA tests and negative reaction with ODC, CIT, $H_2S$, URE, TDA, IND, VP, GEL, SAC, AMY and OX tests.

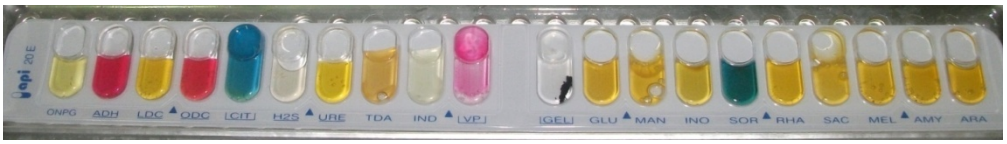


**Photo (B) of E. Sakazakii:** Showing positive reaction with ONPG, ODC , CIT, ADH, VP, GEL , SAC, AMY, GLU, MAN, INO, RHA, MEL and ARA tests and negative reaction with LDC, H2S, URE, TDA, IND, SOR and OX tests.

**Photo (C) of E. Cloacae:** Showing positive reaction with ONPG, ODC , CIT, ADH, VP, SAC, AMY, SOR, GLU, MAN, RHA, MEL and ARA tests and negative reaction with LDC, GEL, INO, H2S, URE, TDA, IND and OX tests.

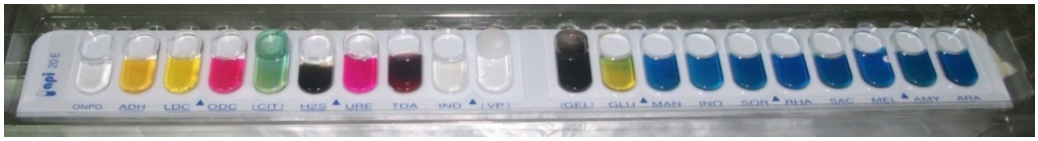


**Photo (D) of Pr.mirabilis:** Showing positive reaction with ODC, H2S, URE, TDA, VP, GEL and GLU tests and negative reaction with ONPG, CIT, LDC, SAC, MAN, AMY, SOR, RHA, MEL, IND, ARA and OX tests.

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