Assiut Vet. Med. J. Vol. 36 No. 72, January 1997

Animal Health Research Laboratory, Assiut
Head of Lab. Prof. Dr. S.M. Nashed

BACTERIOLOGICAL STUDIES ON ENTEROBACTERIACEAE ISOLATED FROM TABLE EGGS IN ASSIUT

(With two Tables)

By

A.F. BASTAWROWS; NAWAL GH. KHALIL; S.R. SEDDEK and SEHAM M. ALY

(Received at 20/12/1997)

دراسات بكتريولوجية عن الميكروبات المعوية المعزولة من بيض المائدة بأسيوط

الفونس فخرى ، نوال غبريال ، صديق رشوان، سهام محمد

أجريت هذه الدراسة على عدد ٢٢٥ بيضة جمعت عشوائيا فى أشهر الصيف من أسواق أسيوط ومحلات البقالة والسوبر ماركت ومنازل الفلاحين ولقد تبين من الدراسة أن ٧١,١١٪، ٤٤,٤٤٪ من قشر البيض ومحتوياته على التوالى تحتوى على الميكروبات المعوية وكان متوسط العدد الكلى للميكروبات المعوية ٤,١×١٠ ٣، ٢,٧×١٠ ٢ لقشر البيض ومحتوياته كذلك تم تصنيف الميكروبات المعوية المعزولة كالتالى. الميكروب العصوى القولونى (أمكن تصنيف ١٢ عتره منها سيرولوجيا)، سالمونيلا تيفى ميوريم، بروتيس (فولجارز، ريتجرى، ميرابلس)، انتيروباكتر (ايروجينز وكلوكا)، كلبسيلا (اوزينى واوكسى توكا)، ستروباكتر (داى فيرسس، فرندای)، سيراتيا ليكوفسينس، بروفيدنسيا. وتناولت الدراسة مناقشة أهمية الميكروبات المعوية المعزولة وعلاقتها بالصحة العامة وكذلك اقتراح السبل الكفيلة بالمحافظة على البيض من التلوث بهذه الميكروبات فى المزارع وأثناء تخزينه وتوزيعه.

SUMMARY

225 eggs were collected in summer months from Assiut city markets, different groceries, supermarkets and farmers' houses and examined for the presence of Enterobacteriaceae. The obtained results revealed that the mean value of total Enterobacteriaceae count / shell and count/ml of egg content samples were $4.1X10^3$ and $2.7X10^2$ respectively. The results also revealed that 71.11% and 44.44% of the examined egg shell and egg content samples were contaminated by Enterobacteriaceae respectively. The different isolates recovered

from the examined samples were identified as *Escherichia Coli* (only 12 isolates could be typed serologically.), *Proteus (vulgaris, rettergi* and *mirabilis*); *Enterobacter (aerogenes* and *cloacae*); *klebsiella* (*ozaenae* and *oxytoca*); *Citrobacter* (*diversus* and *Freundii*); *Serratia liquefaciens Providencia* spp. and *Salmonella typhimurium.* The economic and public health importance of the isolated *Enterobacteriaceae* has been discussed and suggested measures for improving the quality of produced hen eggs are given.

Key words: *Enterobacteriaceae - Table eggs - Assiut*

INTRODUCTION

Family *Enterobacteriaceae* is a large, heterogeneous group of Gram-negative rods whose natural habitat is the intestinal tract of humans and animals. It includes many genera (e.g., *Escherichia, Shigella, Salmonella, Enterobacter, Klebsiella, Serratia, Proteus*) and others. Some enteric organisms are part of the normal flora and incidentally cause disease, while others are regularly pathogenic for man (Brooks et al., 1995).

Since faecal contamination is thought to be a major cause of egg contamination by *Salmonella*, it is not surprising that other members of the *Enterobacteriaceae*, particularly *E.coli*, can also be isolated from eggs (Board and Fuller, 1994). Moreover, Sashihara et al., (1979) found that *Enterobacteriaceae* and *pseudomonas* were the main bacteria in unpasteurized liquid whole egg.

Shell, shell membranes and whole egg contents were screened for members of family *Enterobacteriaceae* by several investigators (Ahmed et al., 1974; Moursy et al., 1982; Ahmed et al., 1985; Ahmed et al., 1987; Alaboudi et al., 1988; El-Essawy et al., 1989; Abd El-Galil et al., 1995 and Lambiri et al., 1995).

Many species of family *Enterobacteriaceae* may constitute a public health hazard. Numerous cases of food poisoning outbreaks were traced to the members of family *Enterobacteriaceae* (Alcamo, 1994; Brooks et al., 1995).

This investigation was planned to assess the enumeration and identification of *Enterobacteriaceae* prevailing on egg shell and egg contents.

MATERIAL AND METHODS

(A) Collection of samples:

225 eggs were collected in summer months at random from Assiut city markets, different groceries, supermarkets and farmers' houses. Every 5 eggs (one group) were placed in a sterile plastic bag and dispatched to the laboratory with a minimum of delay.

(B) Sample preparation:

1) Egg shells:

Egg shells were tested by a surface rinse method as described by Moats, (1979). Each egg was immersed in 100 ml of peptone water in a jar and shaken for 15 minutes on a mechanical rotatory shaker, the rinse solution obtained from the five eggs of each group were mixed. Ten-fold serial dilutions were aseptically prepared from the mixture, using sterile 0.1% peptone water.

2) Egg contents:

The egg samples were handled and prepared to evacuate the content according to Speck, (1976). Each egg was placed on a metal holder, and few drops of 70% ethyl alcohol were poured over the shell at its broad end, ignited and allowed to burn off. A sufficient area of the shell was removed around the air space with sterile scissors. The contents of each group were evacuated aseptically into a sterile container and were mixed using a sterile mixer until the sample became homogenous. Ten-fold serial dilutions were aseptically prepared from the mixture, using sterile 0.1% peptone water.

(C) Experimental techniques:

(1) Enumeration of *Enterobacteriaceae* was carried out on the aforementioned dilutions according to Mercuri and Cox (1979, using violet red bile glucose agar (VRBG). The number of red or purple colonies with diameters >0.5 mm surrounded by a zone of precipitated bile, was counted and the total *Enterobacteriaceae* count per shell and per ml egg contents were then calculated and recorded.

(2) Isolation of Salmonellae.

A heavy inoculum from the rinse solution as well as from the mixed egg contents were transferred to Selenite F broth to be incubated at 37°C for 18 hours. After which loopfuls were streaked

on three specific selected solid medlia (MacConkey, Brilliant green and SS agar). Inoculated plates were incubated at 37°C for 24 hours. Suspected Salmonella colonies (non lactose fermenters) were further identified morphologically and biochemically according to Koneman et al., (1994) and Quinn et al., (1994). Isolates that produced biochemical reactions simulating Salmonella were subjected to serological identification as described by Edward and Ewing (1972) and the instruction of the technical information of the manufacturer laboratory (Anon, 1975). The final decision of typing was made according to Kauffmann white Scheme (Kauffmann, 1972).

(3) Isolation and identification of other members of *Enterobacteriaceae*:-

Each of one mL from the rinse solution as well as, from the mixed egg contents was aseptically inoculated into a tube of MacConkey's broth. The tubes were incubated at 37°C for 24 hours. Loopfuls of the broth were streaked on Brilliant green and MacConkey's agar plates in a way to obtain separate colonies and incubated at 37°C for 24 hours. Different colonies of both lactose and non lactose fermenting bacteria were picked up onto agar slants for further purification and identification according to koneman et. al., (1994) and Quinn et al., (1994).

Serological identification of the isolates that produced biochemical reactions simulating *E. coli*. was carried out after their purification by determination of the "O" and "K" group antigen using slide agglutination test, against the coli antisera obtained commercially from AG, Marburg, W. Germany and following the instruction of the manfacturer.

Other organisms of *Enterobacteriaceae* were identified only by biochemical tests as their respective immune sera were not available.

RESULTS and DISCUSSION

The summarized results in Table (I) verify that 71.11% of marketable egg shell samples were contaminated with *Enterobacteriaceae* in numbers which varied from $1.4X10^2$ to $4.3X10^5$ shell with an average count of $4.1X10^3$/ shell. While 44.44% of egg content samples proved to be contaminated with *Enterobacteriaceae*, the maximum count was $2.0X10^3$/

mL egg content, the minimum was 80/mL egg content with an average count of $2.7X10^2$ /mL egg content.

The high counts met within examined samples are mostly attributed to the unhygienic methods of handling and distribution. Moreover, room temperature storage of eggs where the prevailing climitic condition is very hot in Upper Egypt in summer months. It is apparent that the count of *Enterobacteriaceae* recovered from shells are higher than those from egg content samples as the shell is more liable to be contaminated.

The results given in Table (II) reveal that *E. Coli* was the most prevalent bacteria, the incidence of *E. Coli* was 48.89% and 42.22% of the examined egg shell and egg content samples respectively. Nearly similar results were reported by Ahmed et al., (1974) and Ahmed et al., (1985). On the other hand, our results are significantly higher than reported by Ahmed et al., (1987), Alaboudi et al., (1988) and El-Essawy et al., (1989).

Escherichia coli is taken as an index to indicate recent faecal contamination, as it is a normal inhabitant of the intestinal tract of both man and animals and always present in manure (Garrad, 1946). This organism can grow and penetrate the shell contaminating the contents (Mayes and Takeballi, 1983). Furthermore, *E Coli* is considered one of the main contaminants of deteriorated eggs (Moursy et al., 1982) and certain strains would produce fishy flavours in eggs (Frazier and Westhoff, 1986). Moreover, *E. Coli* may be incriminated in lowering hatchability and early embryonic death in balady hatcheries (Abd El-Galil et al., 1995).

Out of 41 *E. Coli* isolates only 12 strains could be typed serologically, while 29 strains (the remaining strains) were untyped due to lacking of specific antisera. The serovar $^{O}114:{}^{K}90$ was recovered from egg content samples (2 strains, 4.88%) and egg shell samples (1 strain, 2.44%) and the serovar $^{O}86:{}^{K}61$ was recovered with the same frequency, while, the serovar $^{O}119:{}^{K}69$ was only recovered from egg content samples (1 strain, 2.44%). On the other hand, the enteropathogenic serovar $^{O}111:{}^{K}58$ was recovered from egg content samples (5 strains, 12.16%). The enteropathogenic serovars are known to be implicated in several gastrointestinal illness, infantile diarrhoea, food poisoning and other disorders (Kornacki and Marth, 1982). Moreover, Wilson and Miles (1984) reported that *E. Coli* $^{O}111:{}^{K}58$ possessed an adhesive property (Colonization factor) as one of the important virulence factors. Niazi and Refai (1988) demonstrated that *E. Coli* $^{O}111:{}^{K}58$ exhibited not only an adhesive property but was also enterotoxigenic and could produce a heat-labile toxin. The enterotoxigenic *E. Coli* (ETEC) are now considered as

common causes of traveller's diarrhoea and/or infantile illness and gastrointestinal illness (Brooks et. al., 1995). On the other hand, (ETEC) strains had been shown to possess O antigens of various serovars, not all of which are included among the so-called classical enteropathogenic serovars of *E. Coli* (Orskov et. al., 1976). The isolation of at least 5 typed ETEC strains from the content of 225 eggs in this study constitutes a great threat to the health of humans especially children and also of unproperly consumed, some individuals, especially athletics drink freshly beaten eggs.

Results recorded in table (II) revealed that *Salmonella typhimurium* was isolated one time (2.22%) from examined egg shells. A lower incidence (0.4 % of 5 thousands egg shells) was reported by El-Agroudy and Awad (1966). Egg shells get contaminated with Salmonellas either as a result of infected oviduct or faecal carriage and the organism will die rapidly but survival is enhanced by high relative humidity and lower temperature during storage (Board and Fuller, 1994). Dipping such contaminated eggs twice in a 6% hydrogen peroxide solution (which did not impair hatchability), reduced the average number of organisms in egg shell membranes by 95% and the number of *Salmonella typhimurium* positive eggs by 55% as compared with the infected untreated group (Padron, 1995).

Out of 45 egg content samples *Salmonella typhimurium* was isolated from 2 cases with an incidence of 4.45% (Table, II). Nearly similar results were reported by Prost and Riermann (1967) and Akhtar et. al., (1982), while higher incidence was obtained by Moursy *et. al.*, (1982). On the other hand, lower incidences of *Salmonella typhimurium* (0.08% and 2.86%) were obtained by El-Agroudy and Awad, (1966) and Ahmed et. al., (1987) respectively.

Egg contents can become contaminated with Salmonella as a result of either infection in reproductive tissue or the passage through the shell, of organisms derived from either intestinal tract or the environment. Contaminated water may act as an effective route of transmission (Dhillon et. al., 1974), where Salmonella find their way from alimentary tract via blood to ovaries (Gordon and Tuker, 1965). Board and Fuller (1994) reported that when eggs are broken, Salmonella present on egg shells may contaminate the contents and grow rapidly if storage is at ambient temperature.

From the public health point of view, Salmonella infection as well as food poisoning outbreaks have been attributed to consumption of hen eggs or egg products such as whole egg custard, mayonnaise, egg salad and egg nog (Alcamo, 1994). Steere et. al., (1975) concluded that both epidemiological and microbiological evidences indicated that raw egg beaten

in milk (egg nog) was responsible for the infection by *Salmonella typhimurium*.

From the results recorded in Table (II) it is clear that marketable egg content samples contain *Proteus vulgaris, Proteus rettgeri* and *Proteus mirabilis* with an incidence percentages of 4.44%, 8.89% and 2.22% respectively. Nearly similar results were reported by Akhtar et. al., (1982) and Ahmed et. al., (1987), while higher isolation rate of Proteus organisms were recorded by Becirevic (1983) and Ibeh and Izuagbe (1986).

The incidence of *Proteus rettgeri* recovered from shell egg samples was 15.56%, while *Proteus vulgaris*, and *proteus mirabilis* were not isolated (Table II); a lower incidence of Proteus spp. (9.0%) was obtained by Alaboudi et. al., (1988).

It had been stated that Proteus organisms contaminate the egg contents frequently (Alaboudi et. al., 1988), and they had been isolated from spoiled eggs by several investigators (Board and Board, 1968; Ahmed et al., 1974; Moursy et al., 1982). Proteus organisms cause economic losses through deterioration of eggs even when kept at low temp. (Fraizer and Westhoff, 1986). Moreover, Proteus organisms had been incriminated in cases of summer diarrhoea in children and urinary tract infection. Furthermore, in Egypt, Mostafa et al., (1948) reported that 30 out of 125 food borne outbreaks were due to Proteus organisms.

Enterobacter spp. (*Enterobacter aerogenes, Enterobacter cloacae*); Klebsiella spp. (*Klebsiella ozaenae, Klebsiella oxytoca*), Citrobacter spp. (*Citrobacter diversus, Citrobacter freundii*), Serratia spp. (*Serratia liquefaciens*) and Providencia spp. were recovered from examined samples (egg shells and egg contents) at varying percentages ranging from 2.22% to 28.89% (table II). These findings agree to a certain extent, with those reported by several authors (Ahmed et al., 1974, Becirevic, 1983, Ibeh and Izuagbe 1986, Ahmed et al., 1987, Alaboudi et al., 1988 and El-Essawy et al., 1989).

It has been definetely established that such contaminants enter the eggs after they have been laid and that pentration rate is accelerated when the shells are stained with contaminated materials while the eggs are still worm (Jull, 1984).

From the economic point of view *Serratia* spp., *Klebsiella* spp. and *Enterobacter* spp. were implicated in different types of objectionable changes rendering the eggs unfit for human consumption (Board and Board, 1968). Moreover, *Enterobacter coloaca* had been considered as a secondary invador producing spoilage of eggs (Frazier and Westhoff, 1986).

Furthermore, some of isolated strains were claimed to be incriminated in food poisoning and urinary tract infections and occasionally other infections and were often resistant to antimicrobial therapy (Wyah, 1992 and Brooks et al., 1995).

Realizing that the contents of newly laid eggs from a healthy fowl, are usually sterile, and that the rate of contamination of produced eggs depends mainly on the hygienic measures adopted in the farm or during handling and storage of eggs, thus proper farm hygiene, handling and storage are necessary for obtaining eggs of good quality. The practice of cleaning eggs by washing sanitizers is common in egg industry nowadays. Pasteurization of egg products has been introduced as a statutory requirement in many countries.

REFERENCES

Abd El -Galil Y., EL- Kenawy A.I., El-Gmiey S.R. and Abd El-Latif M.M. (1995): Bacterial causes of lowering hatchability and early embryonic chicken deaths in balady hatcheries in Dakahila Governorate. Assiut Vet. Med. J. 33(66): 199-206.

Ahmed, A.A., Abu-Gabal, M.S.M. Enab, S.N. and Moustafa, T.H. (1974): Investigation of microbial spoilage of hen eggs.Assiut Vet. Med. J., 1(1&2). 76-84.

Ahmed, A.A-H., Moustafa, M.K., Aboul-Khier, F. and El-Bassiony, T.A. (1985): Bacterial contamination of egg shells. Assiut Vet. Med. J. 14(27): 122-127.

Ahmed A.A.H., Nagah S.M. and Moustafa M.K. (1987): Microbial contamination of market hen eggs. Assiut Vet. Med. J. 18(36): 124-131.

Akhtar, F. Afzal, H., Ashfaque, M. and Nasim, Z. (1982): Egg shell contamination of human importance with reference to Salmonellae. Pakistan Vet. J. 2(1): 10-11. Cited from Vet. Bull., 52(9): 776: (1982).

Alaboudi, A.R., Hammed, D.A. and Ali, D.S. (1988): Microbial content of market eggs. Indian Jounal of Animal Sciences. 58(7): 769-770.

Alcamo I.E. (1994): Fundamentals of microbiology 4 th ed. The Benjamin/Cummings publishing company, Inc. P. 729-730.

Anon, (1975): Serological identification of Salmonella. Difco Laboratories, Deteroit, Michgan, U.S.A. O168.

Becirevic, M. (1983): Study of the transferable drug resistance of Gram negative bacteria isolated from eggs. Veterinaria, Yugoslavia, 32(4) : 457-472.

Board, P.A. and Board, R.G. (1968): A diagnostic key for identifying organisms from rotten egg. Brit. Poult. Sci. 9:111-120.

Board, R.G. and Fuller, R. (1994): Microbiology of the avian egg. 1st ed. Chapman and Hall P. 94-112-128.

Brooks G.F., Butel J.S., Nicholas Ornston L., Jawetz E., Melnick J.L. and Adelberg E.A. (1995): Medical Microbiology 20 th ed. Prentice-Hall International Inc., P. 206-217.

Dhillon, A.S., Maurer, A.J., Deibel, R. H. and Haller, R.W. (1974): Feeding of different levels of Salmonellae to chickens. Indian J. Poult. Sci. 9:103-107.

Edward, P.R. and Ewing, W.H. (1972): Identification of *Enterobacteriaceae* 3rd. ed. Burgeon publishing Company, Minneapolis Atlanta, U.S.A. 208-337.

El-Agroudy, M.A. and Awad, F.I. (1966): Salmonella in hen eggs. J.Vet. Sci. U.A.R. 3(1): 1-4.

El-Essawy H.A., Saudi A.M. and Sallam S.S. (1989): Microbiological studies on market hen eggs. Alex. J. Vet. Sci., 5(2): 219-225.

Frazier, W.C. and Westhoff, D. C. (1986): Food microbiology 6th reprint Tata McCraw Hill publishing Co. Ltd. New Delhi.

Garrad, E.H. (1946): Coliform Contamination of eggs. Cand J.Research 24 (C): 121-125.

Gordon, R.F., and Tuker (1965): The epizootology of *Salmonella menstom* infection of fowls and the effect of feeding poultry food artificially infected with Salmonella. Brit. Poult. Sci., 6:251-264.

Ibeh, I.N. and Izuagbe, Y.S. (1986): An analysis of the microflora of broken eggs used in confectionery products in Nigeria and the occurrence of enterotoxigenic Gram-negative bacteria. International J.Food Microbiology. 3(2): 71-77.

Jull, M.A. (1984): Poultry Husbandry. Tata McGraw- Hill, Book Company, Inc. New York

Kauffmann, F. (1972): Serological diagnosis of Salmonella species. Kauffmann-white scheme. Munksgaard, Copenhagen.

Koneman, E,W., Allen, S.D., Janda, W.M., Schrecken-berger, P.C. and Winn, W.C. (1994): Introduction to diagnostic microbiology 4 th ed. J.B. Lippincott Company., P. 41

Kornacki, J.L. and Marth, E.H. (1982): Food borne illness caused by *E.coli*, A review. J. Food. prot., 45-1051.

Lambiri, M., Mavridou, A., Richardson, S.C. and Papadakis, J.A. (1995): Isolation of Salmonellas from animals, food, animal feeds and the environment during 1985-1990 in Greece. Acta Microbiologica Hellensca 40 (4): 297-302.

Mayes, F.J. and Takeballi, M. A. (1983): Microbial contamination of the hen's egg: A review J.Food prot. 46(12): 1092-1098.

Mercuri, A.J. and Cox, N.A. (1979): Coliforms and *Enterobacteriaceae* isolates from selected foods. Journal Food Protection 42 (9): 712-714.

Moats, W.A. (1979): The effect of Washing eggs under commercial conditions on bacterial loads on egg shells. Poultry Sci., 58: 1228-1233.

Mostafa, M.N.E.D., Elyan, A. and Gohar, M.A. (1948): J. Egypt. Med. Assoc. 31:556 cited after Reimann, H(1969): Food-borne infection and intoxications Academic press, New York and London.

Moursy, A.W. Al-Ashmawy, A. M. and Moursy, E.A. (1982): Microbiological studies on deteriorated hen eggs. Assiut Vet. Med. J. 9 (17&18): 91-96.

Niazi, Z.M., and Refai, M. (1988): Isolation of Enteropathogenic and Enterotoxigenic *Escherichia coli* from meat and cheese. Vet. Med. J. 36(1), 121-134.

Orskov, F., Orskov, I, Evans, D.J., Sack, R.B., Sack, D.A. and Wadstrom, I. (1976): Special *Escherichia Coli* serotype among Enterotoxigenic strains from diarrhoea in adults and children. Med. Microbiol. Immunol., 162, 73-80.

Padron, M. (1995): Egg dipping in hydrogen peroxide solution to eliminate *Salmonella typhimurium* from egg shell membranes. Avian Diseases. 39(3): 627-630.

Prost, E. and Riemann, H.(1967): Food-borne Salmonellosis. Annual Review Microbiology 21:495-528.

Quinn, P.J., Carter, M.E., Markery, B.K. and Carter, G.R. (1994): Clinical Vet. Microbiology. Year book. Wolfe publishing . Europ limited. P. 209-236.

Sashihara, N., Mizutani, H., Takayama, S., Konuma, H., Suzuki, A and Imai, C. (1979): Bacteriological survey on raw material of liquid (Frozen) whole egg, its products and manufacturing processes "studies on bacterial contamination of liquid (Frozen), whole egg". Journal of Food Hygienic Society. 20(2): 127-136.

Speck, M.L. (ed) (1976): Compendium of methods for microbiological examination of food. American public health Association, Washington, D.C.

Steere, A.C. Hall, W.J. Wells, J.G., Graven, P.J., Leotsakis, N., Farmer, J.J. and Gangarosa, E.J. (1975): Person-to-person spread of *Salmonella typhimuriun* after a hospital common source outbreak. Lancet, 1(7902): 319-322.

Wilson, G.S., Miles, A. and Parker, M.T. (1984): Acute enteritis. Cited by Gross, R.J. (1984): P. 458-476 in Toply and Wilson's principles of Bacteriology, Vicrology and Immunity 7th ed., Vol.3. Edward Arnold (Publishers) Ltd.

Wyah, G.M. (1992): Immunoassays for food poisoning bacteria and bacterial toxins. 1st ed., Chapman and Hall.

Table (I): Statistical analytical results of total Enterobacteriaceae count per shell and mL of examined marketable eggs.

Category	No. of groups "Samples" examined	No. of positive samples		Enterobacteriaceae count/shell or content		
		No.	%	Min.	Max.	average
Egg shells	45	32	71.11	$1.4X10^2$	$4.3X10^3$	$4.1X10^3$
Egg contents	45	20	44.44	80	$2.0X10^3$	$2.7X10^2$

* Positive samples = samples proved to contaminated with *Enterobacteriaceae*

Table (II): Incidence and frequency distribution of *Enterobacteriaceae* organisms recovered from marketable eggs.

No. of groups "Samples" examined	Isolates	Marketable egg shells				Marketable egg contents			
		Positive samples		Isolates		Positive samples		Isolates	
		No.	%	No.	%	No.	%	No.	%
45	**A- Coliforms:-**								
	Escherichia coli	22	48.89	22	27.5	19	42.22	19	45.24
	Enterobacter aerogenes	7	15.56	7	8.75	3	6.67	3	7.15
	Enterobacter cloacae	8	17.78	8	10.00	2	4.44	2	4.76
	Klebsiella ozaenae	13	28.89	13	16.25	4	8.89	4	9.52
	Klebsiella oxytoca	5	11.11	5	6.25	2	4.44	2	4.76
	Citrobacter diversus	2	4.44	2	2.5	-	-	-	-
	Citrobacter freundii	5	11.11	5	6.25	1	2.22	1	2.38
	B- Non-Lactose fermenters								
	Salmonella typhimurium	1	2.22	1	1.25	2	4.44	2	4.76
	Proteus vulgaris	-	-	-	-	2	4.44	2	4.76
	Proteus rettgeri	7	15.56	7	8.75	4	8.89	4	9.52
	Proteus mirabilis	-	-	-	-	1	2.22	1	2.38
	Serratia liquefaciens	6	13.33	6	7.50	1	2.22	1	2.38
	Providencia species	4	8.89	4	5.00	1	2.22	1	2.38
Total				80	100.00			42	99.99