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DIAGNOSIS OF *ENTEROBACTER SAKAZAKI* FROM SAMPLES OF INFANT MILK, STOOL AND HABOUBI HOSPITAL ENVIRONMENT IN DHI QAR PROVINCE AND STUDY THE SENSITIVITY FOR SOME ANTIBIOTICS

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ABSTRACT

This study was aimed for detection occurrence of *Enterobacter sakazakii* in stool specimens, infant formula and hospitals environments. A total of 310 samples (50 infant formula, 10 stool samples and 20 hospital environment swabs), during the period from November 2014 to April 2015 were collected. Growth on Violet red bile agar and MacConkey agar was identified by cultural and biochemical tests and confermated by API 20 E system which revealed that: Only `12/80 (15%) gave positive growth for *Enterobacter* spp. as following: 3/20 (15%) environmental swabs, 8/50 (16%) infant formula and 1/10 (10%) stool samples. Diagnosis of the isolates appeared that *E. cloacae* was (6 /12) while *E. sakazakii* (4 /12) and *E. aminogenus* (2/12). Four *E. sakazakii* isolates were screened for their antibiotic resistance against 20 antibiotics of different classes using Kirby-Bauer disk diffusion method. The results showed that all the tested isolates were resistant to at least 6 antibiotics of which they were tested.

KEYWORDS: *Enterobacter sakazaki*, Infant milk, Stool, Sensitivity, Antibiotics**.**

INTRODUCTION

Enterobacter sakazakii is an opportunistic pathogen that can cause neonatal meningitis, necrotizing enterocolitis and septicemia. These bacteria received increase attention as food

borne pathogens after an outbreak of meningitis in Tennessee in 2001 (Iversen and Forsythe, 2003).

Urmenyiand Franklin, (1961) reported on the first incidence of *E. sakazakii* infections of two fatal cases of neonate meningitis that occurred in England. *E. sakazakii* is a gram negative, peritrichous, motile, non-spore-forming, facultative anaerobic (Oonaka *et al*., 2010).

E. sakazakii has been isolated from a wide variety of food sources (milk, cheese, meats, vegetables, rice, fermented bread, dried foods, herbs, and spices). Powdered of infant formula and milk powdered are the most common vehicles implicated in *E. sakazakii* infections (Osaili and Forsythe, 2009). Clinical outbreaks of infection in neonatal intensive care units associated with contaminated infant milk formula (Block *et a*l ., 2002).

The source of *E. sakazakii* and vehicle of transmission is not always clear however infant formula has been epidemiologically implicated as the source of *E. sakazakii* in several clinical cases (Pinar *et al*., 2009). There are several modes of transmission for these organisms, including exogenous, such as fecal oral, person-person, mother-child,food, hospital equipment, and personnel, and endogenous, from the patient'sown intestinal flora. Passive carriage on the hands of medical personnel constitutes (Dumen, 2010).

The organisms develop persistence to antibiotics by any of the following mechanisms selection, mutation, phage transduction and transference. Microbial resistance can be either hereditary in organism or acquired through the environment (Ibezim, 2005). aim of study The present study was planned for identification of *E. sakazakii* from various specimens from contaminated infant formula and hospitals environment in Thi-Qar province with Study of the Antibiotic susceptibility pattern to *E.sakazakii*.

METHODS

1. Sterilization of media

The media have been sterilized by autoclave at 121 º C and pressure 1.5 bar for 15 min. The glass wear have been sterilized by dry heat in electric oven at 180 º C for 2 hrs.

2. Preparation of culture media

media used in this study were prepared according to manufactures instructions Oxoid, England (Carry Blair media, MacConkey broth, Violet Red Bile Agar, MacConkey agar,

Kliglar Iron agar, Simmon citrate test, Mueller-Hinton Agar, Motility sulfide medium, Peptone water and Methyle red – Voges proskuer broth).

3. Collection of Samples

Atotal of 50 infant formula samples were collected randomly from preterm infants wards and local markets of Thi-Qar province. (FDA, 2002a, b) (with some modification) A total of 10 stool samples were collected from infants with severe diarrhea in Al- haboubi Hospital. All samples were put in Carry Blair media and brought to the laboratory for analysis. (Kim *et al.*, 2008) (with some modification). total of 20 swabs were collected from hospitals environment (Floors, walls and equipments) at Thi-Qar province. The collected swabs were brought to the laboratory for analysis (Bond and Sehulster, 2004).

4. Identification of bacterial isolates

use biochemical tests Growing on Kliglar Iron agar, Catalase test ,Oxidase production (MacFaddin , 2000), Simmon citrate test , Indol test, Methyl red test, Voges- Proskaur (VP) test , Motility test (Collee *et al*., 1996) then Identification of *Enterobacter* spp. By kits API 20 E system the procedure adopted was following the manufacturer's instructions. (BioMerieux/France) was used in this study.

5. Antibiotics susceptibility testing

The antibiotics susceptibility testing was done by the discs diffusion method as that described by Bauer *et al*.,(1966) The turbidity of growing broth culture was adjusted with sterile broth to obtain concentration optically comparable to the 0.5 MacFarland standards tube (growth equivalent to 1.5 $X10^8$ cell/ml).The diameter of growth inhibition zones were measured by using transparent ruler. compared with the standard inhibition diameter of the CLSI (2007). The antibiotic discsused in this study where from Bioanalyse ,Turkey are list in Table (1).

Table (1) Antibiotic discs used

NO	Antibiotic	Concentration µ g	NO.	Antibiotic	Concentration µ g
1-	Amikacin	30	11-	Ciprofloxacin	5
2-	Amoxicillin/Clavulanic	20/10	12-	Gentamacin	10
3-	Ampicillin	10	13	Imipenem	10
4-	Aztreonem	30	14	Levofloxacin	5
5-	Cefepime	30	15	Nalidixic acid	30
6-	Cefotaxime	30	16-	Nitrofurantoin	300
7-	Cephalothin	30	17-	Norfloxacin	10

8-	Ceftazidime	30	18-	Piperacillin	100
9-	Ceftriaxone	30	19-	Ticarcillin	75
10	Chloramphenicol	30	20-	Ticarcillin/Clavulanic	75/10

6- Statistical analysis was done using the SPSS program. Associations between categorical variable were tested by the chi-square test P-value < 0.05 was considered Statistically Significance.

RESULT

1. Isolation and identification of *Enterobacter spp*.

Out of 80 samples (50 infant formula, 10 stool and 20 hospital environments) have been collected and tested during November 2014 to April 2015. Only 12 (15%) samples were given growth for *Enterobacter spp.*, 3 (25%) environmental samples, 8 (66.7%) infant formula and 1 (8.3) stool samples.

2. Primary diagnosis

A- Morphological properties

Colonies of *Enterobacter spp*. On MacConkey agar are muocoid , lactose fermenter, pink color with dark center; some of the isolates (*Enterobacter sakazakii*) produced yellow pigment on TSA after 48hrs at 25 ˚C. Colonies of *Enterobacter spp*. On VRBA are muocoid, pale color except *E. aminogenus* are dry, pink colony. (Fig 1)

Fig (1) *Enterobacter* spp. On VRBA agar A- *E. sakazakii*, B –*E .aminognus*, C- *E. cloacae*

B- Biochemical tests:. Biochemical tests results for the isolates are shown in

Table (2): Biochemical tests used for identification of *Enterobacter spp*.

Bacteria	Kia	Gas.	H_2S	Ind.	MR	VP	Cit.	Ca.	Ox.	Mo.
E. cloaca	A/A	+	-	-	-	+	+	+	-	+
E. aminogenus	A/A	+	-	-	-	+	+	+	-	+
E .sakazakii	A/A	+	-	-	-	+	+	+	-	+

(+) positive ; (-) negative ; (KIA) Kliglar iron agar, (K) alkaline ; (A) acid, (Ox) Oxidase , (Mo) Motility, (Ca) Catalase, (Cit) Citrate,(Ind) Indol,(MR) Methyl red,(VP) Vogues – proskuer

3. Identification by using API 20 E system

All the isolates have been tested by API 20E system for confirmation of the identification and for determination the species. The results showed that of 12 isolates 6 (50%) as *Enterobacter cloacae*, 4 (33.3%) as *Enterobacter sakazakii* and 2(16.7%) as *Enterobacter aminogenus. (Fig.* 2*)*

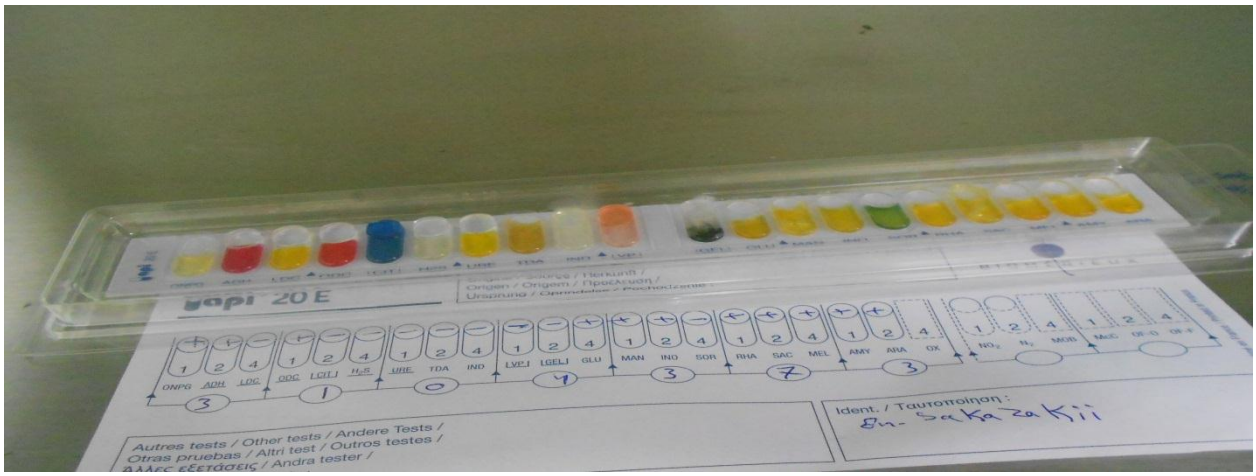

Fig. (2) Reaction of *E. sakazakii* in API 20E.

4. Isolation of *Enterobacter* spp.

A. Isolation of *Enterobacter* spp. from infant formula

Out of 50 samples, 8 (16%) gave positive growth of *Enterobacter spp*. 4(50%) isolates appeared as *E. cloacae* and 2 (25%) as *E sakazakii* and 2 (25%) as *E. aminogenus* which appeared as the lowest percentage of *Enterobacter spp.* isolated from infant formula (Table 3). Statistical analysis showed no significant differences between the bacteria isolated from infant milk. The level of probability ($P \geq 0.05$).

Table (3): The number and percentage of bacterial isolation from infant formula specimens.

Bacteria	Isolation No.	%
E. cloacae	4	(50)
E. sakazakii	2	(25)
E. aminogenus	2	(25)
Total	8	(100)
$X^2 = 2$		

B. Isolation of *Enterobacter spp*. from hospitals environment.

A total of 20 hospital environmental swabs were collected from different regions of the main hospitals at Thi-Qar Province. The results showed that 3(15%) gave positive growth and identified as: 2 (66.7%) *E. cloacae* and 1(33.3%) *E. sakazakii* (Table 4). Statistical analysis showed no significant differences between the *Enterobacter spp.* isolated from environmental samples the level of probability ($P \geq 0.05$).

Table (4) Isolation of *Enterobacter spp.* from environmental samples.

Site of swab	No. of swabs	Isolation of *Enterobacter*		Isolation of *E. cloacae*		Isolation of *E. sakazakii*	
		No	%	No	%	No	%
Bed of patient	5	0	(0)	0	(0)	0	(0)
The operations surgical tools	4	0	(0)	0	(0)	0	(0)
Sinks	3	0	(0)	0	(0)	0	(0)
Patient room floor	4	1	(25)	1	(100)	0	(0)
Patient room walls	2	1	(50)	1	(100)	0	(0)
Patient tables	2	1	(50)	0	(0)	1	(100)
Total	20	3	(15)	2	(66.7)	1	(33.3)
$X^2 = 1.33$							

C. Isolation of *Enterobacter spp*. From diarrheal cases of infant

A total of 10 stool specimens from diarrheal cases of infant (ages less than one year) were tested. The results showed that only one sample was positive for E .*sakazakii* with a percentage of (10%).The positive sample was taken from male infant of 3 month age.

4.3. Antibiotic Susceptibility Pattern

Susceptibility of 4 isolates of *E. sakazakii* against 20 antimicrobial agents from different classes has been determined using Kirby-Bauer disk diffusion method by measuring the diameter of inhibition zones around the antibiotic discs according to CLSI, (2007).

Table (5) is showing that 100% of the isolates were resistant to ampicillin, ceftazidime, Ticarcillin,Ticarcillin/Clavulanic acid, cephalothin and Cefotaxime, 75% were resistant to Amoxicillin/Clavulanic acid . Low resistance were appeared to Nitrofurantoin, Nalidixic acid ,Cefepime and Gentamicin 25% while no resistance appeared to Piperacillin, Aztreonam, Ceftriaxone, Chloramphenicol, Ciprofloxacin , imipenem, Norfloxacin, Levofloxacin and Amikacin. 100% of *E. sakazakii* isolates were showing multidrug resistance. Statistical analysis showed no significant differences between resistance of *E. sakazakii* to some of the antibiotics the level of probability ($P \geq 0.05$).

Table (5): Percentage of antibiotics resistance of *E. sakazakii* against 20 types of antibiotics according to CLSI 2007. (n=4).

Type of antibiotic	Resistant Isolates		Intermediate Isolates		Sensitive Isolates	
	No.	%	No.	%	No.	%
Ampicillin	4	(100)	0	(0)	0	(0)
Piperacillin	0	(0)	2	(50)	2	(50)
Ticarcillin	4	(100)	0	(0)	0	(0)
Ticarcillin/Clavulanic	4	(100)	0	(0)	0	(0)
Amoxicillin/Clavulanic	3	(75)	1	(25)	0	(0)
Cephalothin	4	(100)	0	(0)	0	(0)
Cefotaxime	4	(100)	0	(0)	0	(0)
Ceftazidime	4	(100)	0	(0)	0	(0)
Ceftriaxone	0	(0)	2	(50)	2	(50)
Cefepime	1	(25)	0	(0)	3	(75)
Imipenem	0	(0)	0	(0)	4	(100)
Aztreonam	0	(0)	0	(0)	4	(100)
Gentamicin	1	(25)	0	(0)	3	(75)
Amikacin	0	(0)	0	(0)	4	(100)
Nalidixic acid	1	(25)	2	(50)	1	(25)
Ciprofloxacin	0	(0)	0	(0)	4	(100)
Levofloxacin	0	(0)	0	(0)	4	(100)
Norfloxacin	0	(0)	0	(0)	4	(100)
Nitrofurantoin	1	(25)	2	(50)	1	(25)
Chloramphenicol	0	(0)	0	(0)	4	(100)
Resistant Isolates	$X^2 = 9.9$					

Table (6) show multidrug resistance Isolates

Isolates	Sources of isolates	Type of antibiotic resistance
E.sakazakii	Environmental	More than three antibiotic
E.sakazakii	Infant formula	More than three antibiotic
E.sakazakii	Infant formula	More than three antibiotic
E.sakazakii	stool	More than three antibiotic

DISCUSSION

1. Isolation and Identification of *E. sakazakii*

In the present study, a total of 80 swabs were cultured, only 12 (15%) gave *Enterobacter spp*. This percentage is considered higher than it in a previous study (Mordi and Hugbo, 2011). Another study showed prevalence of *Enterobacter spp.* in UTI (7.1%). (Prakash and saxena, 2013a).

The isolates of *Enterobacter spp*. were distributed as: 3(25%) from hospitals environmental swabs, 8(66.7%) from infant formula specimens, 1 (8.3%) from stool specimens. These results improved the wide distribution of *Enterobacter* spp. (Paterson *et al.*, 2005)

The identification of the 12 isolates is in agreement with other studies (Man *et al*., 2001), that *E. cloacae* is the dominant species (50%) followed by *E. sakazakii* (33.3%) and *E. aminogenus* (16.7%). Another study (Al- Tawfiq *et al*.; 2009) showed that *E. cloacae* was 60% of total *Enterobacter* isolates.

Infant formula which became the main and important source for infant feeding was found contaminated by bacteria. In the present study. 8 / 50 (16%) of infant formula was found contaminated with *Enterobacter spp*., mostly with *Enterobacter cloacae*. This result was documented the results of Fauziah *et al.*, (2008) and Oonaka *et al*., (2010). Also this result is in agreement with Iverson and Forsythe,(2004a) for the isolation of *E. sakazakii* from infant formula.

One of the most striking findings in present study was the isolation of *E. sakazakii*, the most common bacterial species isolated from PIF product over the world (Drudy D , et al ;2006 : Gurtler JB, et al ; 2005) Further, there have been many recalls of *E.sakazakii*-contaminated infant formula in the United States. In November 2002, a nationwide recall of more than 1.5 million cans of dry infant formula contaminated with E. sakazakii was reported (Gurtler JB, et al; 2005) Although one isolate of E. sakazakii was cultured from one sample of formula powders this might have been due to an unequal distribution in the powder or its presence at such a low concentration that it escaped detection by conventional methods. So large amounts of powdered substitutes for breast milk should be obtained from different sources to be analyzed for the presence of this bacterium (Al-Timimi. B J, 2007).

2. Antimicrobial Susceptibility Pattern

In this study, 4 isolates of *E. sakazakii* were screened for antibiotic resistance against 20 antimicrobial agents of different classes using Kirby – bauer method. Reports from 1960-1999 of antibiotic susceptibility of *E. sakazakii* indicate the organism is typically susceptible to ampicillin, tetracycline, chloramphenicol, gentamicin, and the third-generation cephalosporins. Stock and Wiedemann (2002) studied the specific antibiotic profiles of *E. sakazakii* strains. Interestingly, no natural resistance to cephalosporins was detected in wild-type populations of *E. sakazakii*, and these strains appear to lack β -lactamases (Stock and Wiedemann, 2002). In the present study isolates appeared with 100% resistant to ampicillin, ceftazidime, ticarcillin, ticarcillin /Clavulanic, cephalothin and cefotaxime. A previous study showed resistance was 28.8%, 71.4 % respectively for ampicillin and cephalothin (Zhou *et al.,* 2011). Another study showed resistance 100% and 66.7% for ceftazideme and cefotaxime respectively.(Saeed and Musallan, 2011).

The isolates showed high resistance to the antibiotics Amoxicillin/clavunic acid, Nitrofurantoin, Nalidixic acid, Cefepime and Gentamicin and appeared sensitive to pipracillin, ceftriaxone, Chloramphenicol, Ciprofloxacin and Amikacin these result was agreement with the results of Saeed and Musallan, (2011). While in other study showed the *E. sakazakii* more sensitive to Nalidixic acid, Cefepime and Gentamicin and resistante to pipracillin, ceftriaxone, Chloramphenicol (Zhou *et al.*, 2011). The results of this study are in agreement with a previous study which showed no resistance for Imipenem (Prakash andSaxena,2013a,b). Carbapenems are highly stable to β-lactamase hydrolysis, and porin penetration is facilitated by general size and structure. Their susceptibility to most strain of Enterobacteriaceae makes them usefull as treatment for multidrug resistance organism; carbapenem resistance is currently rare among Enterobactericease (Paterson, 2006).

Our results are similar to results of another study which showed high sensitivity to ciprofloxacin levofloxacin and Norfloxacin (Aigbekaen and Oshoma, 2010). Quinolone resistance in Enterobacteriaceae is usually due to alteration in target enzymes (DNA gyrase and/or topoisomerase IV) or to impaired access to the target enzymes, occurring either because of changes in porin expression or because of efflux mechanisms. (Hooper, 1999). The results of this study indicate a high rate of resistance to antimicrobial agents of bacterial strains isolated from PIF products, and this may indicate a widespread antibiotic resistance

among bacteria isolated from different sources, including that of anthropological and environmental origin. (Al-Timimi. B J, 2007)

REFERENCES

1. Aigbekaen, B.O. and Oshoma, C.E. Isolation of *Enterobacter sakazakii* from powdered foods locally consumed in Nigeria. Pak. J. Nutr., 2010; 9: 659-663.
2. Al-Tawfiq, A.; Antony, A. and Abed, M. Antimicrobial Resistance Rates of *Enterobacter* spp.: A Seven years Surveillance Study. Med. Princ Pract., 2009; 18: 100-104.
3. Al-Timimi BJ. Antimicrobial resistance of bacteria isolated from powdered infant formulas (PIF). Medical Journal of Babylon., 2007; 4; .3(4): 255.
4. Bauer, A.; Kirby, W. Sherris, J. and Turtch, M. Antibiotic susceptibility testing by standardized single disk method. Am. J. Clin. Path., 1966; 43: 493-96.
5. Block, C.; Peleg , O.; Minster, N.; Bar-Oz, B.; Simhon , A.; Arad , I. and Shapiro, M. Cluster of neonatal infections in Jerusalem due to unusual biochemical variant of *Enterobacter sakazakii*. Eur J Clin Microbiol. Infect. Dis., 2002; 21: 613-616.
6. Bond, W.W. and Sehulster, L.M. (2004). Microbiological culturing of environmental and medical –device surface. In: Isenberg, H.D.; Miller, J.M. and Bell, M. eds.Clinical microbiology procedures handbook, section 11.Washington, DC: American Society for Microbiology Press.
7. CLSI: Clinical and Laboratory Standards Institute. (2007). Performance standards for antimicrobial susceptibility testing, seventeenth informational supplement, CLSI document M100-S17, Wayna, PA. USA.
8. Collee, G.; Faser, G. ; Marmion, B. and Simmons, A. (1996) . Mackie and McCartney. Practical Medical Microbiology. 14th ed. Churchill Livingstone, New York.
9. Drudy D, Mullane NR, Quinn T, Wall PG, Fanning S. 2006. *Enterobacter sakazakii*: an emerging pathogen in powdered infant formula. Clin Infect Dis., 2006; 1; 42(7): 996-1002.
10. Dumen, E. *Cronobacter sakazakii* (*Enterobacter sakazakii):*Only an Infant problem Kafkas Univ Vet Fak Derg., 2010; 16(Suppl-A): 171-S178.
11. Fauziah, T.; Norrakiah, A. S.; Uma Priya, K. and Norizan, J. (2008).Detection of *Cronobacter (Enterobacter) sakazakii* and Enterobacteriaceae in Powdered Infant Formula and Childrens milk. Proceeding of the Seminar on Food Biotechnology: Perspectives, Challenges and opportunities, pp. 352-360.

12. Food and Drug Administration FDA, (2002a).Isolation and enumeration of *Enterobacter sakazakii* from dehydrated powdered infant formula.
13. Food and Drug Administration FDA, (2002b). Questions and answers on method for *E. sakazakii* in powdered infantformula.
14. Gurtler, J.B.; Kornacki, J.L. & Beuchat, L.R. *Enterobacter sakazakii*: a coliform of increased concern to infant health. International Journal of Food Microbiology., 2005; 104: 1-34.
15. Hooper D.C. Mechanisms of fluoroquinolone resistance updates., 1999; 2: 38-55.
16. Ibezim, E.C. Microbial resistance to antibiotic. African J. Biotechnol., 2005; 4(13): 1606-1611.
17. Iversen, C. and Forsythe, S. Risk profile of *Enterobacter sakazakii*, an emergent pathogen associated with infant milk formula. Trends Food Sci. Technol., 2003; 14: 443–454.
18. Iversen, C. and Forsythe, S.J. Isolation of *Enterobacter sakazakii* and other Enterobacteriaceae from powdered infant formula milk and related products. Food Microbiology., 2004; 21: 771–776.
19. Kim, J.; Cho, S.; Park, Y.; Lee, J.; Kim ,J.; Lee, B.; Lee, H. and Chae, H. Surveillance of Stool Samples for the Presence of *Enterobacter sakazakii* among Korean people .Yonsei Med. J., 2008; 49: 1017-1022.
20. MacFaddin, J. (2000). Biochemical tests for identification of medical bacteria. Lippincott Williams & Wilkins. Philadelphia, USA.
21. Man, P.; Veeke, E.; Leemreijze, M.; Leeuwen, W.; Vos, G.; Anker,J.; Verbugh, H and Belkum, A. Enterobacter species in a Pediatric Hospital: Horizontal Transfer or Selection in Individual Patients ?The Journal of Infectious Diseases., 2001; 184: 211.
22. Mordi ,M. and Hugbo, G. Frequency of isolation of *Enterobacter* Species from avarity of clinical specimens in a teaching hospital in Nigeria Tropical j. of Pharmceutical Res., 2011; 10(6): 793- 800.
23. Oonaka, K.; Furuhata, K.; Hara, M. and Fukuyama, M. Powder infant formula milk contaminated with *Enterobacter sakazakii*. Jpn J Infect Dis., 2010; 63: 103-107.
24. Osaili, T. and Forsythe, S. Desiccation resistance and persistence of *Cronobacter species* in infant formula. Int. J. Food Microbiol., 2009; 136: 214 –220.
25. Paterson, D.L. Resistance in gram negative bacteria: Enterobacteriaceae. Am J Infect Control., 2006; 34: 20-28.
26. Paterson, D. L., and R. A. Bonomo. Extended-spectrum β-lactamases: a clinical update. Clin. Microbiol. Rev., 2005; 18: 657–686.

27. Pınar, E.; Hande ,S.; Gönül ,Ş. and Terken, B. A Hidden Danger in Infant Formulas and Baby Foods: *Enterobacter* sakazakii Contamination FABAD J. Pharm. Sci., 2009; 34: 153-165.
28. Prakash, D. and Saxena, R.S. Antimicrobial Susceptibility Pattern of human pathogenic bacteria related to Enterobacteriaceae family causing urinary tract infection. Advances in Appl. Sci. Res., 2013; 4: 98-104.
29. Prakash, D. and Saxena, R.S.(2013b).Distribution and Antimicrobial Susceptibility Pattern of Bacterial Pathogen Causing Urinary Tract Infection in Urban Community of Meerut City, India. ISRN Microbiol.
30. Saeed, A. and Mussallam, M. First report on *Enterobacter sakazakii* from Sudanese patients. African J. of Microbiol. Res., 2011; 5: 2374-2379.
31. Stock, I. and Wiedemann, B. Natural antibiotic susceptibility of *Enterobacter amnigenus, Enterobacter cancerogenus, Enterobacter gergoviae and Enterobacter sakazakii* strains. Clin. Microbiol. Infect., 2002; 8: 564–78.
32. Urmenyi, A. M. and White-Franklin, A. Neonatal death from pigmented coliform infection. Lancet i., 1961; 313–315.
33. Weir, E. (2002). Powdered infant formula and fatal infection with *Enterobacter sakazakii*. Can. Med. Assoc. J., 2002; 166: 1570.
34. Zhou, x.; Gao, J.; Huang, Y.; Fu, S. and Chen, H. Antibiotic resistance pattern of *Klebsiella pneumonia* and *Enterobacter sakazakii* isolates from powdered infant formula. African J. of Microbiol. Res., 2011; 5(19): 3073-3077.