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The distribution and characterization of Staphylococci in infant foods

MONIKA SHARMA AND YOGENDRA KUMAR

Food poisoning microbial agent can cause diarrhoel disease and ill-health in infant. Staphylococcal organisms constitute a very important group of pathogens which is the great significance because these are the most common bacteria causing food poisoning. Being Iquitos in nature, staphylococci are usually present in raw milk and some strains may turn out to be exterotoxigonic *S.aureus*. The current investigation revealed significance variations in the staphylococcal counts of infant food. A total eight brands of infant milk food and three brands of weaning food samples were collected around from Meerut city in U.P. in the year 2009-10. The collected samples were analysed for staphylococcal counts and their characterization. Staphylococci were recovered from almost all brands of infant foods. Among positive samples, approximately, 3.28 per cent contained more than 5000 staphylococci per gram, while, 11.47 per cent had a count between 501 and 5000 and further observed that 26.23 per cent were considered to be of good quality as they contained less than 10 staphylococci per gram of samples. During the present study, 142 isolates of staphylococci were recovered from infant food samples. All these isolates produced catalase and fermented glucose anaerobically based on additional characters of production of coagulase, phosphatase and anaerobic fermentation of mannitol, 53 strains were identified as *S.aureus*. Therefore, there is a likelihood of incidence of these organisims in the finished products. The chance contamination during processing cannot also be ignored, because these are the most common bacteria causing food poisoning.

Key Words : Food poisoning, Infant food, Staphylococcal, Weaning food, Diarrhoel, Diseases

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INTRODUCTION

In terms of food safety, infant and children are considered to be a part of the high-risk group of individuals as their immune system may have not yet be fully developed (Abdullah *et al.,* 2013). Breast milk feeding is highly perfect food in the first six months of human lives (Joan, 2012). It is a sterile complex nutritional fluid contains antibodies, enzymes, long chain fatty acid and hormones and other necessary nutrient required for infants healthy growth and development (United Nations Children's Fund, 2011). However, there are instances where expressed breast milk is not available at all and where quantity is insufficient and infant formulas serves as substitute. Also, at instances of maternal death before the baby is one year. Infant formulas serves as best alternatives. Some working mother who are employed outside their homes find it difficult to practise exclusive breast feeding and have adopted infant food as supplement to convenience. Fashion crave to maintain breast shape restrain some modern females from breast feeding (Rajput *et al.,* 2009). Milk is a compulsory part of daily diet for the expectant mothers as well as growing children. Milk is valued because it is an important source of many of

MEMBERS OF RESEARCH FORUM

Author for correspondence :
YOGENDRA KUMAR, Department of Animal Husbandry and Dairying, Kisan (P.G.) College, Simbholi, HAPUR (U.P.) INDIA
Email : dryogendrakumarkd@gmail.com

Associate Authors' :
MONIKA SHARMA, Department of Animal Husbandry and Dairying, Kisan (P.G.) College, Simbholi, HAPUR (U.P.) INDIA

the nutrients essential for the proper development and maintenance of the human body (Bashar and Malek, 2006). Milk is a highly nutritious food that serves as an excellent growth medium for a wide range of microorganisms. The microbiological quality of milk and dairy products is influenced by the initial flora of raw milk, the processing conditions and post-heat treatment contamination (Ahmed and Anwar, 2006). Pathogenic bacteria in milk have been a major factor for public health concern since the early days of the dairy industry. Many diseases are transmissible via milk products. Traditionally raw or unpasteurized milk has been a major vehicle for transmission of pathogens (Kessel *et al.,* 2004). Powdered infant formulas and feed products have been associated with some pathogenic bacteria, the etiology of some serious infants microbial infections, illness and death (Shadlia *et al.,* 2008). These formulas are made of dairy products, cereals, fruits and nuts which may contain some micro-organism or become contaminated during manufacturing process.

Food borne microbial agents can cause diarrhoeal diseases and ill-health in infants (Brown *et al.*, 1989; Motarjemi *et al.*, 1993 and World Health Organization, 1989). Worldwide, diarrhoea is the second leading cause of death in children (after neonatal disorders) (Black *et al.,* 2003) and is a leading cause of growth-faltering and malnutrition (Hoppner e*t al.,* 1972). A great proportion (~70%) of diarrhoeal episodes occur due to food borne pathogens transmitted by unhygienic preparation of foods in households (Hoppner *et al.,* 1972). Malaria, diarrhoea, and pneumonia are the major causes of mortality among children aged less than five years. *Staphylococcus aureus* is the most common organism which is capable of causing food poisoning in a variety of milk product. It is clear that there is a need to adopt a inform and standard manufacturing procedure for there indigenous milk product which are of immense economic importance in country. The microbiological quality of market samples of infant milk food is likely to very to great extent due to unhygienic conditions. Dry infant food are not sterile and could be contaminated with various bacteria including certain pathogens. The aim of this study was to investigate the prevalence of staphylococcal counts in infant foods and to characterize these strains.

METHODOLOGY

A total eight brands of infant milk food (Lactodex, Lactogen, Amul spray, Glaxo, Liver spray, Sapan, Raptakos and Parag) and three brands of weaning food (Cerelac, Farex and Nestum) samples of different brands were collected for microbial analysis around from Meerut city in western U.P. in the year 2009-10. The sample were collected with in a month of manufacture and preserved in a cool and dry place till to analysis of staphylococcal count bacteria.

Analysis method of staphylococcal counts:

The microbiological analysis of the collected samples of infant food was done by following parameters. The infant foods were reconstituted by suspending 50g of sample in 450 ml of ringer solution at 45°C. The diluents was used as per the recommendations of Indian Standards Institution (IS : 1547, 1968). The analysis of staphylococcal counts approximate dilutions of samples was spread over prepared plate of blood agar. The plate was incubated for 24 hours at 37°C. These were also examined by Gram's staining reaction and the suspected staphylococcal colonies were transferred on yeast extract glucose agar slant for further test. Confirmation of staphylococcal was done by there biochemical reaction-sugar fermentation, catalase production, phosphatase production, gelatinase production, haemolysins, salt tolerance and coagulase production. The data subjected to statistical analysis also confirmed significant variations (p/_0.01) in bacterial count among the different branch of infant foods.

Analysis of mean sum of squares and F-value :

The data collected were analysed with the statistical package for social science (SPSS). Mean sum of squares, and F-value (ANOVA) were calculated using the same (SPSS) statistical programmes.

The mean squares are computed by divided the SS by the df. This is aim to the computation of the samples variance which divides the sum of squares by degree of freedom. In fact $MS_T = S^2$.

$$\textbf{Mean sum of squares} = \frac{\textbf{Sum of squares}}{\textbf{Degree of freedom}}$$

The F ratio is then computed by creating a ratio of the between group variance to the within group variance-

$$\mathbf{F} = \frac{\mathbf{MS_A}}{\mathbf{MS_{S/A}}}$$

$$F = \frac{\text{Mean sum of squares (among with brand)}}{\text{Mean sum of squares (within the brand)}}$$

OBSERVATIONS AND ASSESSMENT

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads and Fig. 1 to 9.

Incidence of staphylococci:

A wide variation was noted in staphylococcal count in the samples of infant food analysed (Table 1 to 3).

The average log counts, ranged from 1.1 to 3.1 per gram of the samples tested. However, a maximum log counts 3.6 per gram was found in bands Lactodex. Among the cereal weaning food only brands Cerelac, Farex and Nestum was positive for staphylococci containing an average log counts of 2.2, 1.6 and 1.1, respectively.

The distribution profile indicated the occurrence of staphylococci in as many as 61.00 per cent of samples, as 61 out of a total 100 samples tested, showed the presence of staphylococci. Among the positive samples, approximately 3.28 per cent contained more than 5000 staphylococci per gram, while 11.48 per cent had a count between 501 to 5000 (Table 2). Based on international standards (ICMSF, 1974), majority of these products were of substandard quality because of heavy load of staphylococci per gram. On comparing with these standards, the present observed that 26.23 per cent were considered to be of good quality as they contained less than 10 staphylococci per gram of samples (Table 3). Umoh *et al.* (1985) was analyses one hundred and ten of the 114 sample of infant milk formulae collected from nursing mothers contained viable staphylococci with the highest mean count of 1.0×102 cfu g^{-1}, from samples collected the day the tin was opened (Mathur and Reddy, 1983) reported that very high counts of *S.aureus* in eight samples of infant milk formulae. Cunha-Nelo and A-da, (2002) among tested 37 samples of fresh and kept milk food *Staphylococcus* and *Bacillius cereus* were found as significant variation with regard to incidence of staphylococci was observed among and within different brands. Afroz *et al.* (2013) give the formation of effective levels of toxin requires a high number of micro-organisms (approximately 105-106 micro-organisms per ml of food). In this experiment, staphylococci were found in 6 powder milk samples out of 12 tested samples. It can be concluded that staphylococci were found in almost all brands of infant milk food and weaning food.

Table 1 : Occurrence of staphylococcal in infant foods

Brands	Log counts / g		
	Minimum	Maximum	Average
Lactodex	0.0	3.6	3.1
Lactogen	0.0	2.4	2.1
Amul spray	1.0	2.0	1.7
Galaxo	0.0	0.0	0.0
Liver spray	0.0	2.8	2.5
Spawn	1.3	2.0	1.8
Raptakos	0.0	0.0	0.0
Parag	0.0	2.9	2.6
Cerelac	1.7	2.4	2.2
Farex	0.0	2.0	1.6
Nestum	0.0	1.5	1.1

Table 2 : Distribution of staphylococcal in infant foods

Range (Counts/g)	Positive samples	Per cent
Less than 10	16	26.23
10-100	12	19.67
101-500	24	39.34
501-1000	4	6.56
1001-5000	3	4.92
More than 5000	2	3.28

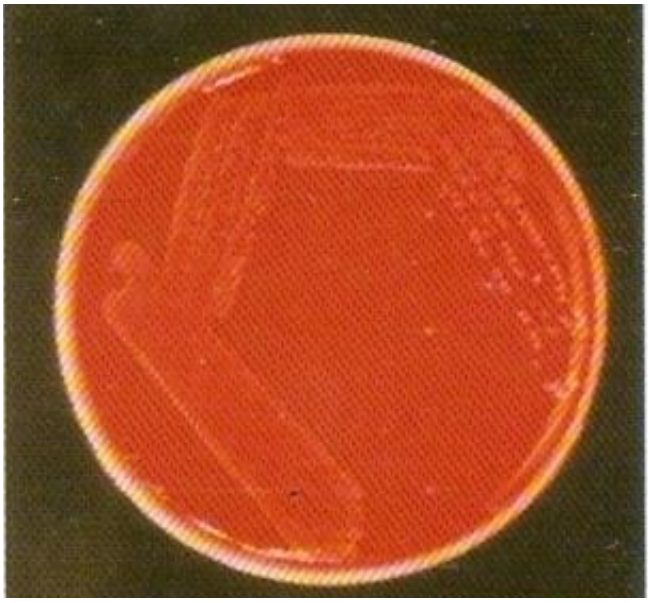


Fig. 1 : Blood agar with golden yellow coloured colonies of *Staphylococcus aureus*

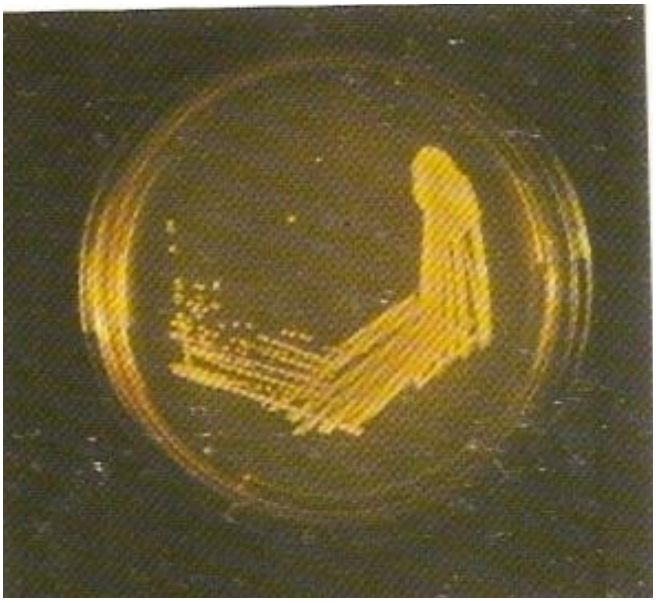


Fig. 2 : Nutrient agar showing yellow Pigmented colories (*Staphylococcus aureus*)

Table 3 : Analysis of variance for staphylococcal counts in infant foods

Source of variation	Degree of freedom	Sum of squares	Mean sum of squares	Calculated F value
Within brands	88	67.09	0.76	6.38**
Among brands	12	58.17	4.85	

**Significant (P/_0.01).

Table 4 : Anaerobic mannitol fermentation and other characteristics of staphylococcal in infant foods

Characteristics	Mannitol fermenters (110 positive samples)		Mannitol non-fermenters (30 positive samples)	
	Number	Per cent	Number	Per cent
Phosphatase	94	85.45	17	56.67
Gelatinase	87	79.09	21	70.00
Salt tolerance (7.5%)	99	90.00	15	50.00
Haemolysins	35	31.82	5	16.67
Coagulase	54	49.09	2	6.67

Characterization of staphylococcal isolates :

Typical staphylococcal colonies on blood agar were golden yellow and in addition a beta type of haemolytic is seen. The colonies were 2-4 mm in diameter, circular, smooth, convex, opaque and easily emulsifiable. Their microscopic examination revealed Gram's positive spherical cell in irregular clusters. Based on these tests, 140 isolates were confirmed as staphylococci. These were catalase positive and fermented glucose anaerobically.

Correlation among different characterization of staphylococcal isolates :

The relationship among different characteristics for the identification of *S. aureus* has been illustrated in Table 4 to 9

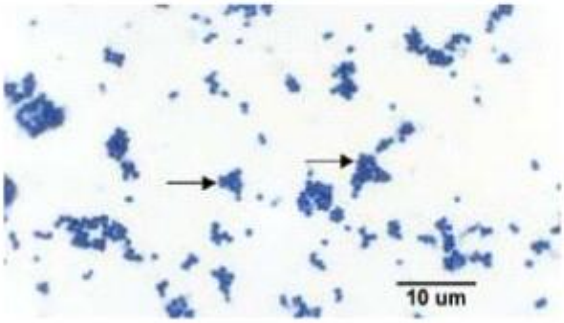
About 85.45 per cent of the anaerobic mannitol fermenters among staphylococcal isolates were positive for phosphatase and 79.09 per cent gelatinase, 90 per cent tolerated 7.5 per cent salt concentration (Table 4). However, 49.09 per cent of such cultures produce coagulase. While, 31.82 per cent produced haemolysins. On the other hand, none of the mannitol non-fermenters produced only about 6.67 per cent were positive for coagulase production. Interestingly, 56.67 and 70 per cent of the mannitol non-fermenters were found to be phosphatase and gelatinase producers and 16.67 per cent were also found to be haemolysins and 50 per cent tolerate

salt at 7.5 per cent level.

Among the phosphatase positive cultures, 87.39, per cent fermented mannitol anaerobically, 78.38 per cent produced gelatinase and 89.19 per cent had normal growth in presence of high salt concentration (7.5%) (Table 5). However, 31.53 and 46.85 per cent of those isolates produced haemolysin and coagulase, respectively. On the other hand, non-phosphatase produced 44.83 per cent mannitol fermenters anaerobically and 72.41 and 51.72 per cent were produced gelatinase and salt tolerant at 7.5 per cent level. Interestingly, 17.24 and 13.79 per cent of the non-phosphatase isolates were found to be haemolysins and coagulase.

It may further be noted that among the gelatinase isolates of staphylococci, 83.33 per cent fermented mannitol (anaerobically) and an 81.48 per cent produced phosphatase. About 34.26 and 46.29 per cent of the gelatinase isolates were produced haemolysin and coagulase, respectively (Table 6). Approximately 83.33 per cent of these isolates were produced salt tolerant (7.5%). On the other hand 9.38 and 18.75 per cent of gelatinase negative isolates also elaborated haemolysin

- Gram-positive
- (0.5 - 1 μm)



- Non-motile, non-sporeforming
- Catalase (+)
- Facultative anaerobes

Fig. 3 : Gram staining of *Staphylococcus*

Fig. 4 : Ferments mannitol

Table 5 : Phosphatase production and other characteristics of staphylococcal in infant foods

Characteristics	Phosphatase producers (111 positive samples)		Phosphatase non-producers (29 positive samples)	
	Number	Per cent	Number	Per cent
Mannitol fermentation (anaerobic)	97	87.39	13	44.83
Gelatinase	87	78.38	21	72.41
Salt tolerance (7.5%)	99	89.19	15	51.72
Haemolysins	35	31.53	5	17.24
Coagulase	52	46.85	4	13.79

Table 6 : Gelatinase activity and other characteristics of staphylococcal in infant foods

Characteristics	Gelatinase producers (108 positive samples)		Gelatinase non-producers (32 positive samples)	
	Number	Per cent	Number	Per cent
Mannitol fermentation (anaerobic)	90	83.33	20	62.50
Phosphatase	88	81.48	23	71.88
Salt tolerance (7.5%)	90	83.33	26	81.25
Haemolysins	37	34.26	3	9.38
Coagulase	50	46.29	6	18.75

and coagulase, while, 62.50, 71.88 per cent of these isolates produced mannitol fermented anaerobically and phosphase, respectively. It was further noticed that 81.25 per cent of these isolates were observed to salt tolerant (7.5%).

Taking salt tolerance as the single criterion to identify different isolates of staphylococci, it may be observed that 83.62 and 78.45 per cent of the salt resistant isolates produced ferment mannitol anaerobically and phosphatase, gelatinase and haemolysins were produced by 73.28 and 31.89 per cent of the cultures. However, only 43.10 per cent of such isolates elaborated coagulase (Table 7). Interestingly, the coagulase staphylococcal isolate had accent per cent relationship with anaerobic fermentation of mannitol and salt tolerance (Table 9). Majority of them produced phosphatase (76.79%) as well as gelatinase (83.93%). While, 32.14 per cent of coagulase positive isolates produced haemolysins.

Among haemolytic isolates of staphylococci, 90 per cent fermented mannitol (anaerobically) and 82.50 per

Fig. 5 : Haemolysis of RBCs

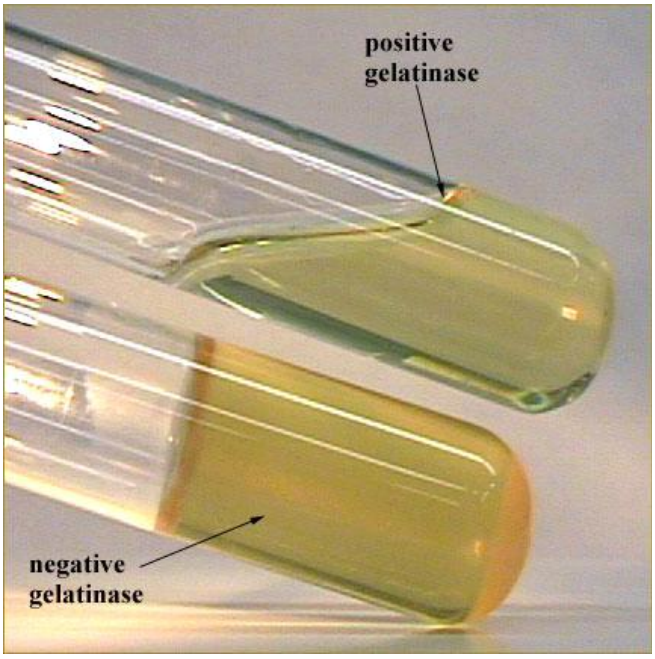


Fig. 6 : Tube gelatinase test

Table 7 : Salt tolerance and other characteristics of staphylococcal in infant foods

Characteristics	Salt tolerant (116 positive samples)		Salt non-tolerant (24 positive samples)	
	Number	Per cent	Number	Per cent
Mannitol fermentation (anaerobic)	97	83.62	13	54.17
Phosphatase	91	78.45	20	83.33
Gelatinase	85	73.28	23	95.83
Haemolysins	37	31.89	3	12.50
Coagulase	50	43.10	6	25.00

Table 8 : Haemolysin production and other characteristics of staphylococcal in infant foods

Characteristics	Haemolysin producers (40 positive samples)		Haemolysin non-producers (100 positive samples)	
	Number	Per cent	Number	Per cent
Mannitol fermentation (anaerobic)	36	90.00	74	74.00
Phosphatase	33	82.50	88	88.00
Gelatinase	26	65.00	82	82.00
Salt tolerance (7.5%)	24	60.00	92	92.00
Coagulase	24	60.00	32	32.00

cent also produced phosphatase. About 60 per cent of the haemolytic staphylococci were observed to synthesize coagulase (Table 8). Approximately, 32 per cent of non-haemolytic staphylococcal isolates also produced coagulase. 65 and 60 per cent of haemolytic isolates were also produced gelatinase and salt tolerant (7.5%). On the other hand, 74 and 88 per cent non-haemolytic staphylococcal were produced mannitol fermenter and phosphatase, respectively. Interestingly 82 and 92 per cent of non-haemolytic isolates were elaborated gelatinase and salt tolerant (7.5%).

On the other hand, 26.19 per cent of the coagulase negative cultures were observed to be positive for haemolysin. A remarkably high proportion of the these coagulase negative isolates produced phosphatase (80.95%) gelatinase (72.62) fermented mannitol anaerobically (80.95%) and salt tolerance (84.52%). Ghosh and Laxminarayana (1974) also found at least 15.38 per cent of dried milk sample to contain staphylococci while, 7.6 per cent had coaglulase positive strains. Bogdanovicova *et al.* (2016) analysis microbiological criteria for coagulase positive staphylococci are upto 101-102 cfug^{-1}. Singh *et al.* (1980) also examined one brand of infant milk food with 9.1×10^{2} organisms also exhibited the maximum number of staphylococci and some these were coagulase positive. Hoppner *et al.* (1972) examined a total of 194 samples of milk used for infant feeding including 75 samples of dried milk formulae were examined microbiologically. About eight samples were found to be contaminated with

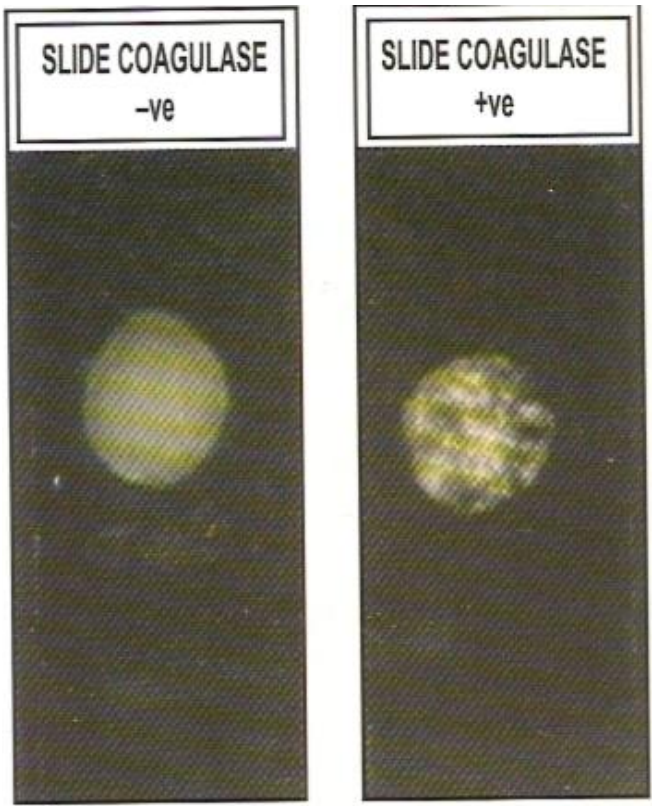


Fig. 7 : Slide coagulase test

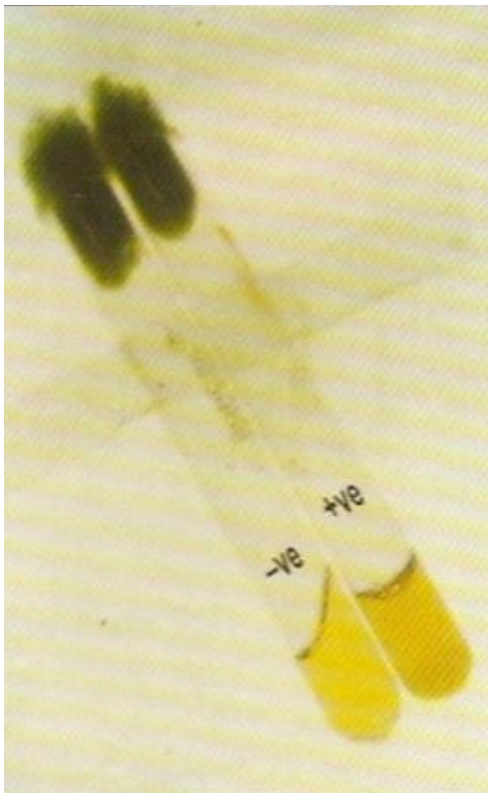


Fig. 8 : Tube coagulase test

Table 9 : Coagulase production and other characteristics of staphylococcal in infant foods

Characteristics	Coagulase producers (56 positive samples)		Coagulase non-producers (84 positive samples)	
	Number	Per cent	Number	Per cent
Mannitol fermentation (anaerobic)	42	75.00	68	80.95
Phosphatase	43	76.79	68	80.95
Gelatinase	47	83.93	61	72.62
Salt tolerance	45	80.36	71	84.52
Haemolysin	18	32.14	22	26.19

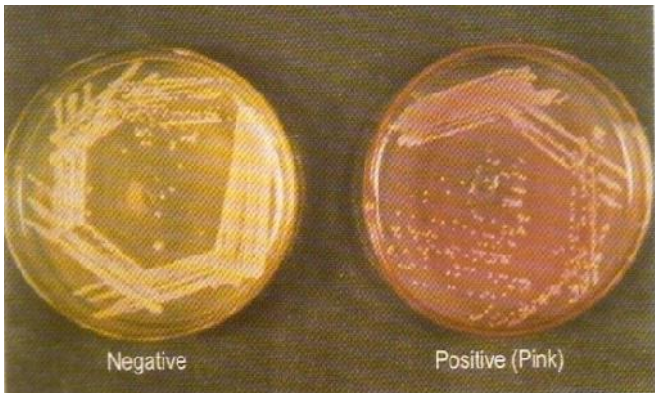


Fig. 9 : Phosphatase test

staphylococcal enterotoxin. Rowan and Deans (2001) studied 47 strains representing fourteen *Bacillus* spp. and Staphylococcus isolates from clinical and milk samples were grown in reconstituted commercially available infants milk formulae. Afroz *et al.* (2013) also found in their experiment staphylococci in six powder milk samples out of twelve testing samples. Wang *et al.* (2012) also confirmed 29 *S. aureus* strain were isolated from powder infant formula milk and 25 from infant rice cereal. It can be concluded from the table that based on the biochemical characters of coagulase production, phosphatase and anaerobic fermentation of mannitol, 53 strains were identified as *S. aureus*.

Conclusion :

It is clear from the results obtained from the experiment that staphylococci were found in almost all brands of infant food and weaning foods. Thus, from the above study it may be concluded that infant milk food and weaning foods can be a potential source of food poisoning with infant and cause serious health problems in babies. Type of incriminating organisms and their toxic metabolites are also important factor affecting the safety of such risk food. There is an urgent need for adoption of international standard such as ICMSF, FAO/WHO for the strict adherence to microbiological quality of these products for delicate and vulnerable infant consumers.

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