STUDIES ON THE BACTERIAL CONTENT OF MILK BEFORE AND AFTER PASTEURIZATION OF MILK SUPPLY SCHEME, PATNA IN RELATION TO PUBLIC HEALTH

Thesis

Submitted to the Faculty of Veterinary Science,
RAJENDRA AGRICULTURAL UNIVERSITY, BIHAR,
in partial fulfilment of the requirements
for the award of degree of
MASTER OF SCIENCE (VETERINARY)
IN
VETERINARY PUBLIC HEALTH & FOOD HYGIENE.

By
Jagata Nand Pandey
B. V. Sc. & A. H. (Hons.)
Post-Graduate Department of Veterinary Public Health & Food Hygiene
BIHAR VETERINARY COLLEGE
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DEDICATED

TO

SRI TRIDANDI SWAMI JEE MAHARAJ

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PATNA,

Dated, the 16th September, 1975.

This is to certify that the work embodied in this Thesis entitled "STUDIES ON THE BACTERIAL CONTENT OF MILK BEFORE AND AFTER PASTEURIZATION OF MILK SUPPLY SCHEME, PATNA IN RELATION TO PUBLIC HEALTH" is the bonafide work of Dr. Jagata Nand Pandey and was carried out under my guidance and supervision.

L.N. Mandal.
16.9.75.

(L.N. MANDAL).

I.C. Gnanarajan
30.10.75
EXTERNAL EXAMINER

C E R T I F I C A T E

Certified that the research work incorporated in this Thesis has not been published in part or in full in any other journal.

Jagata Nand Pandey

(JAGATA NAND PANDEY). 16/9/75

A C K N O W L E D G E M E N T S

The author wishes to record his profound and deep sense of gratitude to his reverned teacher and guide, Dr.L.N. Mandal, B.V.Sc. & A.H., M.Sc. (Vet), P.G., F.R.V.A.C. (Denmark), Ex-Professor of Food Hygiene and Dairy Science and Head of the Department of Veterinary Public Health and Food Hygiene, Bihar Veterinary College, Patna and at present Deputy Director, Animal Husbandry, Central Range, Patna, for suggesting the problem, his benevolent guidance, dexterous supervision, constructive criticism during the course of my research work and help in preparation of the manuscript.

The author wishes to place on record his deep sense of gratitude and indebtedness to Dr. T.S. Sharma, M.S. (Wyoming), Ph.D. (Minnesota), Professor and Head of the Department of Microbiology, Bihar Veterinary College, Patna, for his valuable help and suggestions.

The author is earnestly thankful to Dr. C.R.Prasad, M.Sc.(Vet), F.R.V.A.C. (Denmark), Lecturer, Department of Bacteriology, Bihar Veterinary College, Patna for his valuable suggestion and discussions from time to time.

The author will be failing to fulfil his duties unless, he records his sincere thanks and deep sense of gratitude to Dr. B.N. Sahai, M.V.Sc., Ph.D., Professor and Head of the Department of Parasitology and Veterinary Public Health, Bihar Veterinary College, Patna, for his encouragement and

timely help throughout this endeavour.

The author is highly obliged to Dr. K.N.Tiwary, Ex-Principal, Bihar Veterinary College, Patna, for providing timely and adequate facilities during the period of this work.

The author is indebted to Padmshri S.K.Chakrabarty, I.A.S., Ex-Vice Chancellor, Rajendra Agricultural University, Bihar, Pusa, for selecting him and providing facilities to complete this study as an in-service candidate.

Thanks are also due to the staffs of Veterinary Public Health and Bacteriology Section, for their co-operation during the period of this study.

The author is really indebted to his parents, uncle, wife and children for their hearty co-operation during the period of this study.

My sincere thanks are also due to Dr. B.K.Sinha, M.Sc.(Vet.) and Dr. T.N. Verma, M.Sc. (Vet.), Junior Assistant Research Officers, Disease Investigation, Control and Livestock Production Centre, Bihar, Patna, for their help during this study.

J.N. Pandey
16-9-75

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C O N T E N T S

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INTRODUCTION

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Milk contains all the food constituents required in the human diet in proper proportions. It is an important source of animal protein and fat in India. The importance of milk as a vital source of animal protein of high biological value for the man kind is unquestionable. It is indispensable particularly for vegetarians, infants and the convalscents.

India shares one-fourth of the world's bovine population but paradoxically enough contributes only 6% to the world's milk production (Singh, 1966).

The total annual output of milk in the year 1970-71 was 22.5 million tonnes i.e. about 100 gms per person per day; when shared by 600 millions people. According to experts, the minimum requirement for balanced nutrition is 280 grammes for each individual per day. The per capita consumption of milk in India is only 130 gms as against 741 gms in Switzerland, 637 gms in New Zealand, 623 gms in U.S.A. and 509 gms in England (Singh, loc. cit.).

The importance of milk as food needs no emphasis. Most people are aware of the enormous wastage of milk because of its high perishability. Milk provides an admirable culture medium for bacteria and serves as a vehicle for diseases. It is subject to microbial contamination from the time of its

production till its utilization.

It is easily infected by pathogenic organisms derived from the animals, human personnel and other sources. Such milk is known to communicate several dreadful diseases like typhoid, paratyphoid, brucellosis, tuberculosis, diphtheria, scarlet fever etc. to man kind. The introduction of organisms in milk which may give rise to the production of enterotoxins also leads to human diseases. The toxins are frequently heat stable and are not inactivated or destroyed even by boiling.

Airborne dust and droplets can contaminate milk with organisms such as Staphylococci, Rickettsia and Streptococci which may produce either harmful toxins or direct infections among human consumers.

Further more the danger may be due to some diseased conditions of the animals. Such milk may also get mixed up with huge quantities of wholesome milk and may render the whole lot dangerous.

Dairying in this country is in the developing stage. Intensive efforts are being made for higher milk production. But the production of an abundant, safe and wholesome milk remains as a dream. The problem of hygienic production of milk is a very complex one and be set with great difficulties, more particularly in India, where the general public, milkers, vendors of milk etc. are mostly illiterate and primitive in their knowledge of scientific matters. Milk is open to contamination and wilful adulterations of milk is also one of the causes of contamination

in India. The qualitative aspect of milk production is of great significance to the nation as it is concerned with the maintainance of public health and economic utilization of milk. It is reported that about 7 per cent of total quantity of milk produced in this country is spoiled due to the action of various bacteria (Ranganathan et al., 1964).

Pasteurization was introduced in the country with an aim to render the milk safe for human consumption and to enhance its keeping quality. Heavy initial bacterial contamination along with large number of thermoduric organisms in raw milk hinders in meeting the bacterial count standard for pasteurized milk. Pathogenic organisms have also been reported from pasteurized milk due to faulty handling during and after processing by the various workers. Healthy carriers, both animal and human beings, constantly shed pathogens into milk in the dairy farms and pasteurization plants. Milk requirement of Patna is met by co-operative milk supply Scheme after pasteurization. Very little work has been carried out to assess the bacterial count and the pathogenic organisms per ml of raw and pasteurized milk in Bihar.

Due to the fact stated above the present study has been undertaken on the milk samples from the local Milk Supply Scheme, Patna to assess the microbial flora and the number of pathogenic organisms per ml of raw and pasteurized milk. This study will be helpful in comparing the milk standards of W.H.O. and I.S.I. and to fix up bacterial standard per ml of milk. Here the milk standard is assessed

only on the basis of fat percentage and S.N.F. Hence it was essential to carry out study in regards to bacterial contamination. This will help in fixing up bacterial standard of milk.

The study of microbial flora in pasteurized milk will also help in the supply of wholesome milk to consumers by plugging the deficiency of the pasteurization process if any in the pasteurization plant of Milk Supply Scheme, Patna and in assessing the standard of the pasteurized milk.

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REVIEW OF LITERATURE

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Bacteriological quality of raw milk :

Joshi (1916) for the first time in India, recorded bacterial counts of milk samples to be 9-20 millions per ml drawn from cattle, stables, dairies, milk hawkers and milk shops in Bombay city.

Andresen (1932) reported that paratyphoid bacteria grow very badly in fresh raw milk, whether taken aseptically or not. The inhibitory effect is preserved by efficient and immediate cooling of the milk, but is decreased in proportion to the bacterial content of the milk and is quickly destroyed by heating, a distinct effect being produced by heating at 56°C for 10 minutes.

Kliewe and Herwing (1936) reported the presence of cocci including mastitis - Streptococci in almost all the samples examined.

Barnes (1936) reported that in a particular dairy the average bacterial count was 1600 per ml and individual count varied from 100 to 19000 per ml.

Caserio (1937) observed that the effect of washing the teats with ordinary water or with hot water and soap and the hands of the milker with a nail brush and soap and water as a preliminary to the milking showed a markable reduction in a ratio of about 200 to 1 for example, 148,000 per ml in

the unwashed control, 890 when washing with ordinary water, 620 with soap and water.

Gunnison, *et al*. (1940) recorded the presence of haemolytic *Streptococci* in raw market milk.

Turner and Smith (1941) examined the milk of 16 dairies and found that three of them was infected with haemolytic enterococci. In one dairy the organisms were recovered from the drinking water, in another from both the drinking water and the cow's faeces whilst in the third, water, food and faeces were all negative.

Beahm (1942) recorded the presence of *Streptococci* in the raw milk samples.

Banerjee and Sen (1946), during their studies on bacterial content of milk of the Calcutta Milk Supply Scheme suggested bacterial count of 22 millions per ml. About 97 per cent of the samples showed high coliform content.

Graige (1946) reported 8 strains of Escherichia in the raw milk samples.

Varma (1949) suggested tentative standars for raw milk. He proposed a total bacterial count of about 500,000 per ml and a coliform count below 1,000 per ml for raw milk supplied from an organised dairy farm.

Verma *et al*. (1950) studied the incidence of spore forming aerobes in market milk in the country and encountered *Bacillus subtilis*, *B. cereus*, *B. megaterium*, *B. lentus*, *B. coagulans*, *B. brevis*, *B. sphericus* and *B. stearothermophilus*.

Laxminarayan and Iya (1955) reported 67.77 per cent of total bacterial population of milk due to Micrococci.

Lagrange and Nelson (1961) examined 701 samples of manufacturing grade bulk tank milk and reported one million bacterial count per ml in 37.7 per cent of samples. They also observed more than 10,000 thermoduric micro-organisms per ml in 43.6 per cent of samples.

Galton et al. (1962) obtained 71 per cent coagulase positive Staphylococci in 1,010 milk samples.

Indian Standard Institute (1962) suggested certain standards for grading of raw milk. A standard plate count below 2,00,000 per ml of milk was graded as very good milk, between 2,00,000 and 10,00,000 per ml as good milk, between 10,00,000 and 50,00,000 per ml as fair in quality and milk containing bacteria over 50,00,000 per ml was taken as poor in quality.

Murray (1962) examined raw milk samples in Ireland and reported more than 50 Staphylococcus aureus per ml in 57.7 per cent of samples.

Ranganathan et al. (1964) recorded 13 species of Micrococcus in Indian milk.

Ionescu et al. (1966) investigated the frequency of Bacillus cereus in highly mixed fresh raw milk and reported the organisms in 72.4 per cent samples.

Jones et al. (1966) studied 123 pooled milk samples out of which 77 yielded 83 Mycobacterial strains.

Krishna Mohan and Misra (1967) recovered 33 coagulase positive and 38 coagulase negative strains of Staphylococcus aureus in 200 samples of milk supplied to Patna Milk Supply Scheme.

Ibrahim and Luft (1968) reported higher total and coliform counts in summer and autum than in winter from the market milk of Assiut city.

Jain and Saraswat (1968) studied standard plate, thermoduric and psychrophilic counts in 127 samples of raw milk collected from organised dairy farms, City market and rural collection centres to know the bacteriological quality of market milk in Udayapur city. They recommended standard plate and psychrophilic counts not exceeding one million and thermoduric count not exceeding 50,000 per ml for satisfactory bacteriological quality of milk.

Lavania (1969) graded milk supply of Baraut town (Merrut) on the basis of bacteriological and chemical quality. He collected samples from village milk vendors, individual milk producers, small private dairies and milk collection centres. The respective average values of standard plate counts for these samples were 173.37×10^4, 85.87×10^4, 140.69×10^4, 126.75×10^4 and 176.50×10^4.

Schliesser and Unertt (1970) reported that the cultural examination of 572 milk samples revealed 14.7 per cent of mycobacteria in raw milk samples.

Ninan Thomas and Laxminarayana (1972) during their study on incidence and species distribution of enterococci in

farm and village produced milk at Karnel, reported average standard plate counts of 1,55,00,000 and 8,87,00,000 per ml in farm and village produced milk respectively.

Kumawat et al. (1972) reported to have 2,90,000 to 120 millions per ml for the standard plate, 10 to 5,90,000 per ml for Coliform and 10 to 10,00,000 per ml for the Enterococcus count.

Patro (1973) studied 20 raw milk samples processed for the standard plate count. The total count ranged from 3.5 millions to 300 crores per ml. The average total count was 61.25 crores per ml; and reported the presence of Staphylococcus aureus, S. epidermidis, Micrococcus luteus, Streptococcus lactis, Str. cremoris, Str. faecalis, Str. thermophilus, Str. pneumoniae, Bacillus subtilis, B. sphericus, B. megaterium, B. coagulans, B. circulans, B. stearothermophilus, B. firmus, Alcaligenes faecalis, A. bronchosepticus (Bordetella bronchoseptica), Citrobacter freundii, Escherichia coli, Proteus mirabilis, Providence B group, Pseudomonas aeruginosa, Aeromonas liquefaciens, A. formicans and Pasteurella pseudotuberculosis.

Bacteriological quality of pasteurized milk :

Slanetz (1938) reported that weak haemolytic Streptococci are prevalent in pasteurized milk. They enter the milk at the farm from utensils inefficiently cleaned and sterilized. Samples of raw milk from 10 of the 15 farms studied revealed these Streptococci. They were found to be heat resistant and resist pasteurization temperature. 60 strains of these

Streptococci were examined by the authors. They produced alpha prime type colonies on blood agar and are classified as Streptococcus bovis var A, B, C or D or as Str. faecalis.

Hackler (1939) reported outbreak of Staphylococcus milk poisoning in pasteurized milk.

Banerjee and Sen (1946) reported that pasteurized milk samples collected at Calcutta gave plate counts varying from 3,75,000 to 33 millions per ml.

Sen and Laxminarayana (1948) recorded an average plate count of 1,32,000 in pasteurized milk collected from milk booths of Bangalore city.

Kalkbrenner (1949) carried out 32 examinations of the milk reaching two dairies in a large town after it had been treated and pasteurized. Both dairies employed flash pasteurization plants, at one plant the temperature was raised from 5° to 74°F in about 44 seconds, and at the other from 5° to 85°C in one minute. After being cooled, the milk was irradiated. The result for the treated milks were uniformly bad. Plate counts (48 hours at 37°C) varied from 40,000 to over 7,00,000. Bacterial coli content was over 1,000 per ml in all, with 59 per cent. Over 10,000 Streptococci were found in large numbers in every sample and in over half of them haemolytic colonies were present. Though the author admits that some of the bad results may have been due to faulty pasteurization, he considers that many have been due to subsequent contamination.

Anderson and Meanwell (1950) recorded the influence of unsatisfactory raw milk on the keeping quality of pasteurized

milk. Atmospheric temperature 21°C at the time of the raw milk collection or the inclusion of only 1 per cent of unsatisfactory raw milk in the bulk before pasteurization causes a marked deterioration in keeping qualities.

Olsen (1951) showed the presence of Coliform bacteria in heat treated milk which either indicates insufficient heating, addition of raw milk or contamination after heating.

Williams and Zimmerman (1951) studied the thermal death time in minutes for the vegetative cells at 53°C and the spores at 99.5°C. It was determined for several strains of bacilli including Bacillus cereus, B. subtilis, B.mycoides and B. mesentericus. The results showed that species of aerobic spore forming bacilli, could produce both vegetative cells and spores of unequal heat resistance and the vegetative cell resistance was not related to spore resistance.

Murray (1952) reported Microbacteria, few Streptococci, Spore bearers and miscellaneous types of organisms with Micrococci as dominant one in freshly pasteurized milk.

Galesloot (1953) stated that high plate counts of commercially pasteurized milk were caused by Microbacteria thermoduric Streptococci and non-thermoduric Achromobacteria. Microbacteria originated from improperly sterilized milk cans and Achromobacteria from post pasteurization contamination.

Gartner and Schatzel (1953) noticed marked increases in total bacterial and Coliform counts in pasteurized milk when examined from the cooling section and after bottling.

Storegards (1955) investigated the keeping quality of pasteurized milk in the skane district of Sweden. The bacterial count ranged from 100 to 4,42,000 per ml. He stated that the keeping quality of commercially pasteurized milk entirely depends on the type of contaminating organisms.

Shroff and Bhat (1955) isolated Micrococci from the pasteurized milk collected from the distributing centres in Bombay.

Sinha et al. (1968) studied the keeping quality of commercially pasteurized cow and buffalo milk kept in cold storage and when exposed to warm temperature (37°C). They also recorded the total bacterial and coliform counts and observed a 40 per cent loss in keeping quality in milk when exposed to 37°C for 6 hours.

Vijai and Saraswat (1968) reported 32,000 bacteria per ml.

Ninan Thomas and Laxminarayana (1972) reported 2,70,000 to 28,00,000 and 10 to 200 per ml for standard plate and Enterococci counts in pasteurized milk at Karnel.

Patro (1973) reported that the plate count varied from 3 lacs to 3 crores per ml and the average count was 6.85 lacs per ml in pasteurized milk and investigated the presence of Staphylococcus aureus, S. epidermidis, Streptococcus faecalis, Str. thermophilus, Str. faecium var durans, Bacillus megaterium, B. coagulans, B. stearothermophilus, B. firmus, Citrobacter freundii and Escherichia coli in pasteurized milk samples.

Milk and diseases :

Michael Taylor (1857) reported a number of cases of typhoid fever spreading through milk in England.

Levy (1950) reported an outbreak of diphtheria due to Corynebacterium diphtheriae in Norwegian Municipal Hospital. The raw milk was supplied from the hospital farm where the farmer and his wife were found to be carriers of the causative organism.

Manser and Wilson (1952) described an epidemic of sore throat due to consumption of unpasteurized milk. Three carriers of group A haemolytic Streptococci were detected.

Staack (1953) recorded an epidemic of paratyphoid fever due to infected milk in Schleswig Holstein.

Horstmann (1954) recorded an explosive outbreak of typhoid fever in North Fyn in 1953.

Monterio and Patel (1955) observed two cases of undulant fever in Bombay by consuming contaminated milk.

Seelinger (1961), on the basis of collected evidences from sporadic cases and outbreaks of Listeriosis in Europe, indicated that milk, in many cases, was the vehicle of the organism causing Listeriosis. The organisms causing Listeriosis in man and animals in an area were found to be identical on the basis of serotyping of Listeria monocytogens (Kaplan et al.,1962). From this finding it is inferred that the disease has zoonotic importance and the milk is one of the vehicles of the infection.

Donker - Voet (1965) also considered a cow excreting Listeria in her milk to be a potential source of health hazard.

Mathur (1968) reported a case of Brucellosis due to Brucella melitensis on consumption of improperly pasteurized milk. They also recorded seven family outbreaks of undulant fever near Karnel and one in Karnel town. Both adult and children suffered equally from Br. melitensis. The affected people either took raw infected goat milk or cow and buffalo milk adulterated with infected goat milk or ice cream prepared from such milk.

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MATERIALS AND METHODS

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Collection of materials :

The raw and pasteurized milk samples were collected from the Milk Supply Scheme (Pasteurization plant), Patna, situated near Bihar Veterinary College, Patna. Pooled raw milk samples were obtained from the Milk Supply Scheme, Patna which were received from various adjoining rural collection centres like Maner, Bihta, Bikram and Pali of Patna district.

Pasteurized milk samples were collected in sterile test tubes before bottling from the storage chamber of the pasteurization plant.

Source of samples	Kind of samples	No. of samples	Time of collection.	Remarks
Milk Supply Scheme (Pasteurization plant), Patna.	Pooled raw milk.	40	2 P.M.	
-do-	Pasteurized milk from the plant itself before bottling.	40	4 P.M.	The pasteurized milk was taken from the same lot of pooled raw milk.

Milk samples were collected in sterile test tubes after thorough agitation of the bulk milk. All possible aseptic precautions were taken to avoid external contamination during sampling.

Procedure for standard plate count :

The samples collected under strict precautions were processed in the laboratory on the same day without any further delay. Each sample was thoroughly shaken for uniform distribution of micro-organisms and diluted serially with sterile normal saline solution which was prepared by adding 8.5 gms of NaCl in 1000 ml of distilled water.

One ml of the sample was transferred to the first tube of dilution containing 9 ml of sterile normal saline solution. The contents of the first dilution tube were mixed thoroughly well. Then 1 ml from this tube was transferred to the second tube containing 9 ml of sterile normal saline solution. Again 1 ml from second tube was transferred to third tube containing sterile diluent. This process was repeated to 10^7 dilution in case of raw milk and 10^5 in case of pasteurized milk. Separate clean sterile pipettes and test tubes were used for each dilution.

Total bacterial count in the milk samples were studied by standard plate count technique. 1 ml of the suitable dilution was poured into sterile petridishes and 12-15 ml of nutrient agar, previously melted and cooled at 45-48°C were poured to each petridish. The petridishes were moved in clockwise and anti-clockwise directions and then allowed to solidify after thorough mixing. Then the plates were incubated at 37°C for 24 hours.

Petridishes containing 30-300 colonies of micro-organisms were selected for counting of bacteria per ml.

Procedure for isolation and identification of Micro-organisms:

After the total count the colonies were characterized into different types by their colony characters, morphology of organisms and gram's staining. Each type of separate colonies were transferred into nutrient broth and incubated at 37°C for 24 hours. A loopful of culture from each broth was streaked over nutrient agar for isolation of pure cultures. Then the pure colonies were kept on nutrient agar slants for the sake of identification.

The samples were also inoculated on blood agar, MacConkey agar and tetrathionate broth directly at the time of processing for standard plate count. Blood agar was of great importance in isolating haemolytic Streptococci and different species of Bacillus. MacConkey agar helped in differentiating lactose fermenting bacteria from non-lactose fermenting bacteria.

Identification of the isolates at generic and species level was done with the help of "Manual for the identification of medical bacteria" by Cowan and Steel (1970). "Bergey's manual of determinative bacteriology" by Breed et al. (1957) and "Medical microbiology" by Cruickshank (1965) were also consulted from time to time. Cultural, morphological, staining characteristics and biochemical reactions were the main criteria used for identification of various bacterial species.

To detect the genus of the micro-organisms the following primary tests like shape, presence of spore, motility, ability of the organisms to grow in air, catalse activity, oxidase

production and the method of carbohydrate break down in Hugh and Leifson's (O-F) medium were done.

Catalase activity :

Two to three drops of 3 per cent hydrogenperoxide solution was poured on a clean glass slide then with the help of sterile platinum loop the micro-organism to be tested was taken in very less quantity and kept on the glass slide. Formation of bubbles in the hydrogenperoxide solution indicated the organism to be catalase positive and the absence of bubbles formation indicated the organism to be catalase negative.

Oxidase activity :

One to two drops of 1 per cent tetramethyl-p-phenylenediamine aquous solution was taken on a filter paper. The culture to be tested was taken with the help of a platinum loop and smeared on the area of reagent. A positive reaction was indicated by the appearance of a dark purple colour on the paper within 5-10 seconds.

Hugh and Leifson's test (oxidative fermentative test) :

This test was performed to find out whether the micro-organisms attack on sugar by oxidation or fermentation.

Duplicate tubes of O-F medium were inoculated by stabbing the culture with the help of straight platinum wire.

2-3 cms deep layer of paraffin was added to one of the tubes. Both the tubes were incubated at 37°C for 24 hours.

Yellow colour in both the open and sealed tubes indicated fermentation where as yellow colour in the open tube and green colour in the sealed tube indicated oxidation. Green colour in both the tubes indicated no action on carbohydrate.

Preparation of Hugh and Leifson's (O-F) medium :

Peptone	-	2 gms
NaCl	-	5 gms
K_2HPO_4	-	0.3 gm
Agar	-	3 gms
Distilled water	-	1000 ml
Bromthymol blue (0.2 aqueous solution)	-	15 ml

The solids were dissolved in the water by heating. The pH was adjusted to 7.1. Then the indicator was added to the solution. The medium was sterilized at 10 lb pressure for 20 minutes.

A sterile solution of glucose was added aseptically to the medium to give 1 per cent final concentration. The medium was thoroughly mixed and distributed aseptically in 5 ml volumes into sterile tubes. Addition of one gram excess agar to 1000 ml of O-F medium yielded good result.

The following media were used in the present study and they were prepared as per standard method outlined by Cowan and Steel (1970) :

1. Nutrient broth.
2. Salt broth (6.5% NaCl).
3. Peptone water.
4. MacConkey broth.
5. Nitrate broth.
6. Tetrathionate broth.
7. Koser's citrate.
8. Clark and Lubb's medium (MR-VP medium).
9. Litmus milk.
10. Nutrient agar.
11. Blood agar.
12. MacConkey agar.
13. Hugh and Leifson's (O-F) medium.
14. Gelatin agar.
15. Christensen's urea medium.
16. Blood tellurite agar.
17. Aesculin agar.
18. Triple sugar iron agar.
19. King's agar A and King's agar B.
20. Hippurate agar.

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OBSERVATIONS

OBSERVATIONS

In the course of present study forty raw milk samples and forty pasteurized milk samples of Milk Supply Scheme, Patna (Pasteurization plant unit) were subjected to bacteriological examination for assessing microbial load (total viable count) and types of microflora present in them.

TOTAL VIABLE COUNT.

Pooled raw milk obtained from Milk Supply Scheme, Patna :

The pooled raw milk samples obtained from Milk Supply Scheme, Patna were processed for the total viable count. The total count ranged from 2.52 lacs to 600 millions per ml. The average total count was 18.4 millions per ml.

Pasteurized milk before bottling obtained from Milk Supply Scheme, Patna :

The samples of pasteurized milk before bottling were collected from the pasteurization plant of Milk Supply Scheme, Patna and were processed for the total viable count. The total viable count varied from 18.2 thousands to 10 millions per ml. The average total viable count was 2.74 lacs per ml.

Total viable counts of the milk samples are presented in Table I.

MICROFLORA IN MILK.

All the micro-organisms isolated from the milk samples

were identified mainly on the basis of morphological, cultural and biochemical characters.

Pooled raw milk obtained from Milk Supply Scheme, Patna :

All the pooled raw milk samples were subjected to bacteriological analysis for isolation and identification of bacteria. The following bacteria were isolated and identified:

Staphylococci :

Nineteen strains of Staphylococci were isolated from all the samples.

All the Staphylococci were positive for catalase activity and negative for oxidase activity. They produce acid from glucose and attacked glucose fermentatively in Hugh and Leifson's (O-F) medium.

In the present study, the coagulase production was considered as the property of Staphylococcus aureus and were taken as potential pathogenic Staphylococci. The coagulase negative strains were treated as Staphylococcus epidermidis.

Out of 19 Staphylococci, 6 strains were found to be Staphylococcus aureus on the basis of coagulase production in rabbit plasma. The rest 13 were coagulase negative and were treated as Staphylococcus epidermidis. None of the strains produced haemolysis.

Micrococci :

Altogether 7 strains of Micrococci were isolated, out

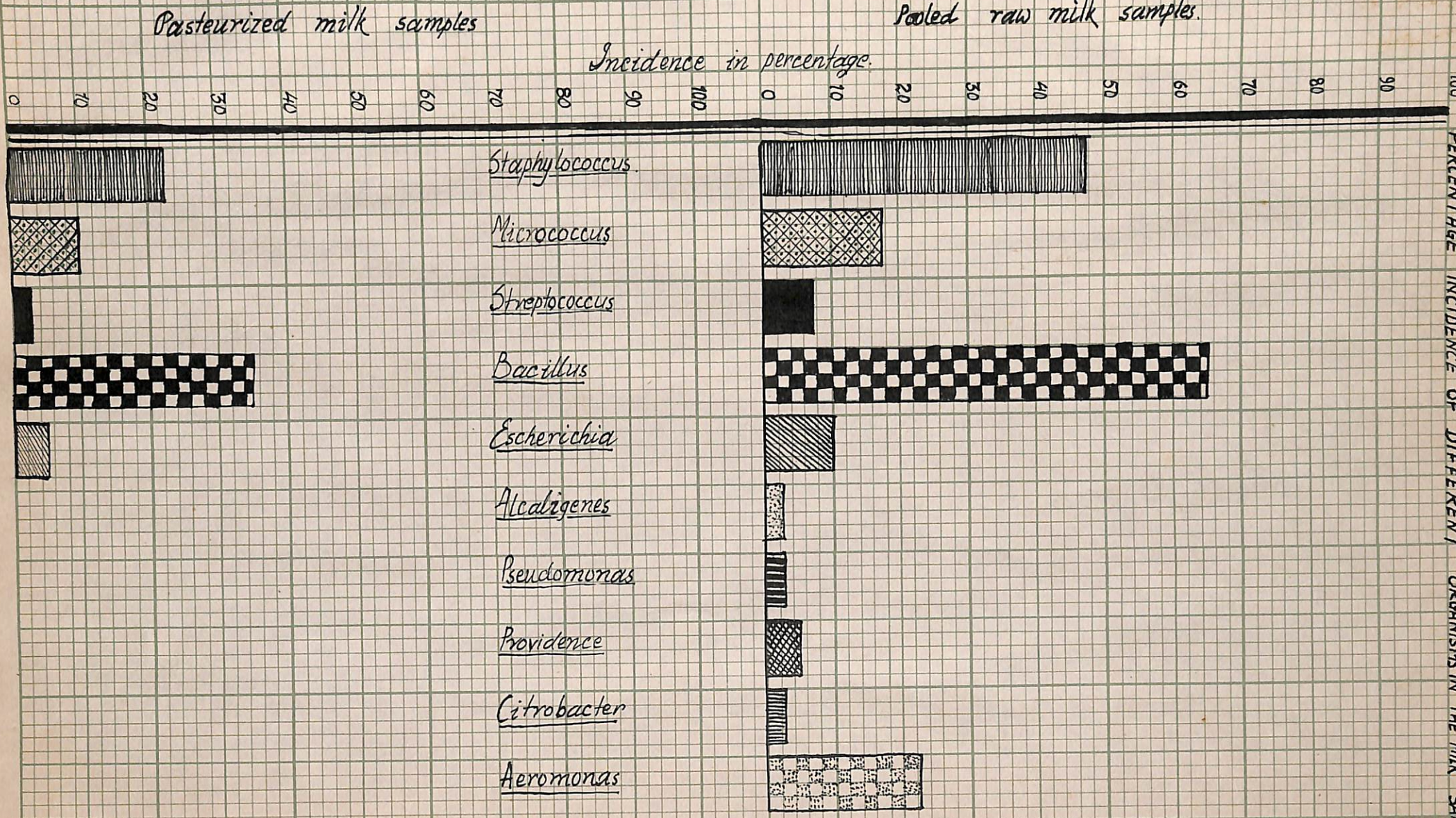


FIGURE - 1

PERCENTAGE INCIDENCE OF DIFFERENT ORGANISMS IN THE MILK SAMPLES.

of which two were found to be Micrococcus roseus and Micrococcus leteus each and 3 strains were identified as Micrococcus (sp.).

The isolates were positive for catalase test and negative for glucose fermentation (O-F) test, V.P. reaction and coagulase activity.

Streptococci :

Streptococcus faecalis was isolated from two milk samples. All the isolated strains grew at 45°C in blood agar and in nutrient broth containing 6.5 per cent NaCl. Acid is produced by the organisms from glucose, maltose, trehalose, salicin, mannitol, sorbitol, sucrose and lactose. Arabinose and raffinose were not fermented. In litmus milk Streptococcus faecalis isolates produced acid, clot and reduced the indicator. The strains do not produce haemolysis on sheep blood agar. The strains hydrolysed aesculin but not hippurate. On MacConkey agar, the strains developed as small pink colonies and black colonies on blood tellurite agar.

Streptococcus lactis :

This was recovered from one milk sample. It does not grow at 45°C in blood agar and in nutrient broth containing 6.5 per cent NaCl. This strain produced acid from glucose, maltose, trehalose, mannitol, sucrose and lactose but not from arabinose, raffinose, salicin and sorbitol. It produced haemolysis in sheep blood agar and hydrolysed aesculin, producing black colour in and around the colonies. When inoculated in litmus milk Streptococcus lactis formed acid, clot and reduced the litmus.

Aerobic spore formers :

Altogether 8 strains of aerobic spore formers were isolated from the pooled raw milk samples of Milk Supply Scheme, Patna. They were Bacillus subtilis, B. cereus, B. alvei, B. firmus, B. lentus, B. brevis, B. coagulans, B. megaterium.

All the isolated Bacilli were motile, spore forming rods and had more or less spreading colonies on nutrient agar.

Eleven milk samples were positive for Bacillus subtilis. These strains uniformly fermented glucose, arabinose, mannitol and sucrose, utilized citrate and hydrolysed gelatin. They were positive for V.P. reaction, nitrate reduction and negative for urea hydrolysis and lactose fermentation.

Bacillus cereus : - Seven samples were positive for Bacillus cereus.

Bacillus alvei : - Two samples were positive for Bacillus alvei.

Bacillus firmus : - One sample was positive for Bacillus firmus. Bacillus lentus, B. brevis, B. coagulans were also found in only one sample.

Bacillus megaterium : - Two samples were positive for Bacillus megaterium.

All the Bacilli isolated from these samples were negative for haemolysis on sheep blood agar.

Escherichia : - Escherichia coli strains were recovered from four pooled raw milk samples. They were gram-negative short rods, motile and non-spore forming.

These strains were positive for indole formation, M.R. reaction and negative for V.P. reaction, citrate utilisation, urease activity and H_2S production. They produced acid and gas from glucose, lactose, arabinose and mannitol but not from sucrose, adonitol, dulcitol and inositol. On blood agar none of the strains produced haemolysis.

Alcaligenes : - Alcaligens faecalis was isolated from only one sample. The isolated strain was gram-negative rod, motile and oxidase positive. They produced acid from glucose. The strain does not fermented maltose, mannitol and lactose. Nitrate was not reduced which was detected by the blackening of the lead acetate paper.

Pseudomonas : - One sample was positive for Pseudomonas aeruginosa.

Providence group : - Two milk samples were positive for Providence B group of organisms. They were gram-negative motile rods and attacked sugar fermentatively in Hugh's and Leifson's medium. Providence isolate fermented glucose and inositol. Other sugars like lactose, arabinose, sucrose, adonitol, dulcitol and mannitol were not fermented.

Citrobacter : - Citrobacter freundii was isolated from one pooled raw milk sample. The recovered strain was gram-negative short rods, motile and non-spore former. It produced acid and gas from glucose, lactose, arabinose, sucrose,mannitol and dulcitol. Adonitol and inositol was not fermented,citrate was utilized. This strain was positive for M.R. reaction and hydrogensulphide production in Triple sugar iron agar and negative

for indol formation, urease activity and haemolysis.

Aeromonas : - Aeromonas liquefaciens were isolated from 9 pooled raw milk samples. They were gram-negative motile rods, oxidase positive and attacked glucose fermentatively in O-F medium.

Aeromonas liquefaciens isolates utilized citrate and hydrolysed gelatin. They produced acid and gas from glucose and acid from lactose, mannitol and sorbitol. Indol formation, V.P. reaction, nitrate reduction were positive in all the strains. Urease activity was negative. Haemolysis was observed in sheep blood agar in all the strains. Isolates were non-pigmented.

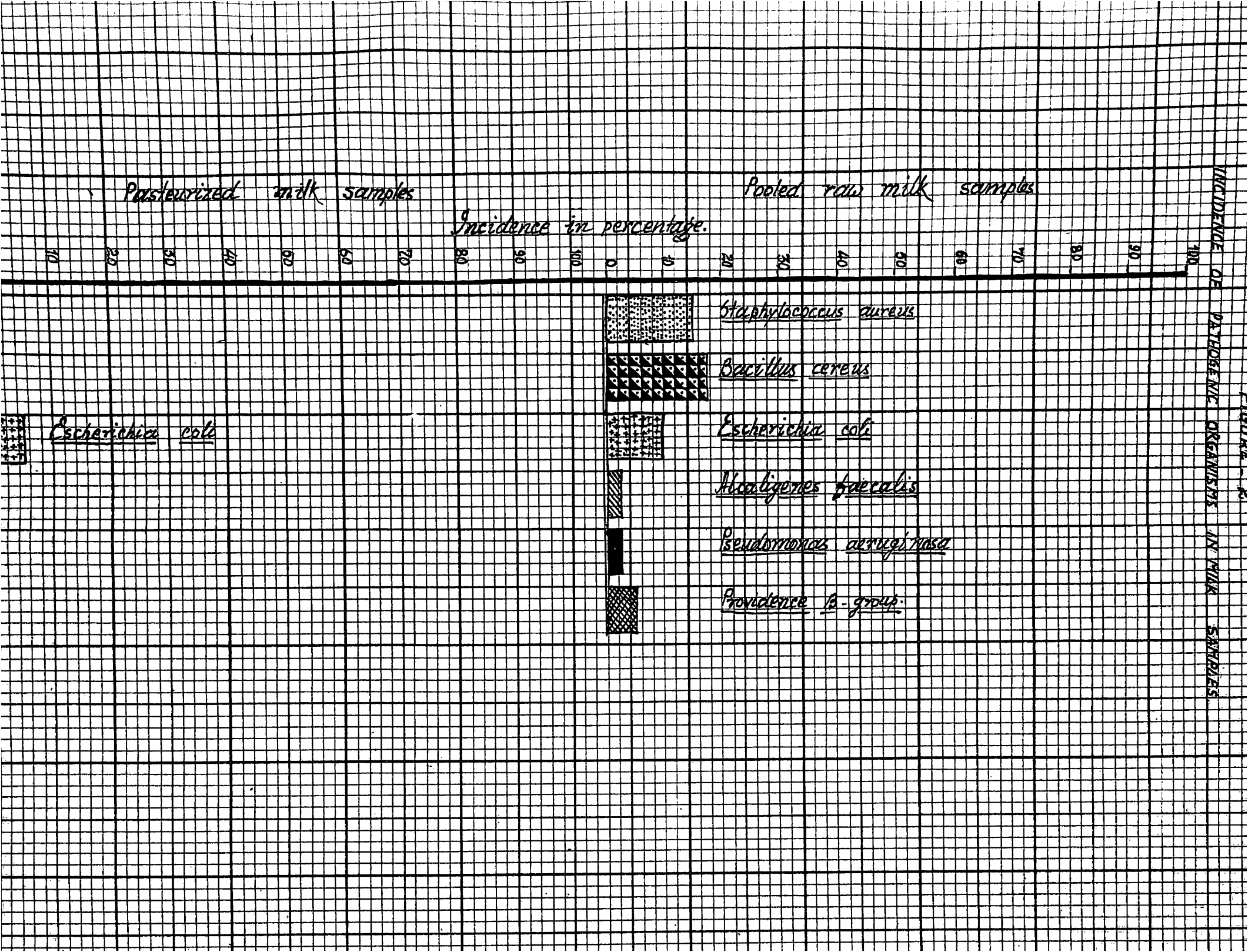
MICROFLORA IN PASTEURIZED MILK:

Pasteurized milk samples were collected from the pasteurization plant of the Milk Supply Scheme, Patna just before bottling and were examined for the micro-organisms.

Staphylococci : - Altogether nine strains of Staphylococcus epidermidis were isolated out of 40 pasteurized milk samples.

Micrococci : - Two pasteurized milk samples were positive for Micrococcus roseus and two strains were identified as Micrococcus (sp.).

Streptococci : - In this study one sample was positive for Streptococcus lactis and another one for Streptococcus faecium var durans.


Pasteurized milk samples
Pooled raw milk samples
Incidence in percentage.
10
20
30
40
50
60
70
80
90
100
0
10
20
30
40
50
60
70
80
90
100
Escherichia coli
Staphylococcus aureus
Bacillus cereus
Escherichia coli
Alcaligenes faecalis
Pseudomonas aeruginosa
Providence A-group.
INCIDENCE OF PATHOGENIC ORGANISMS IN MILK SAMPLES

Streptococcus faecium var durans developed on blood agar at 45°C and in nutrient broth containing 6.5 per cent NaCl. It fermented glucose, maltose and lactose. The strain produced acid in the litmus milk and hydrolysed aesculin.

Streptococcus lactis : - This strain did not grow at 45°C in blood agar and in nutrient broth containing 6.5 per cent NaCl. The strain produced acid from glucose, maltose, trehalose, mannitol, sucrose and lactose but not from arabinose, raffinose, salicin and sorbitol. When inoculated in litmus milk, Streptococcus lactis isolate formed acid, clot and reduced the litmus. It produced haemolysis in sheep blood agar.

Aerobic spore formers : - Bacillus megaterium, B. subtilis, B. alvei, B. coagulans, B. lentus and B. firmus were recovered from the pasteurized milk samples.

Out of forty pasteurized milk samples two samples were positive for Bacillus megaterium, six samples for Bacillus subtilis, two samples for Bacillus alvei, one sample for Bacillus coagulans and Bacillus lentus each and two samples for Bacillus firmus.

Escherichia : - Only two strains of Escherichia coli were isolated from all the pasteurized milk samples. They were non-haemolytic.

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TABLE - I

Standard plate count of milk samples

Sl.No. of samples	Standard plate count per ml	
	Pooled raw milk of Milk Supply Scheme, Patna	Pasteurized milk just before bottling
1	81×10^5	190×10^3
2	146×10^4	267×10^2
3	197×10^4	194×10^3
4	58×10^5	125×10^3
5	136×10^4	35×10^5
6	259×10^4	150×10^4
7	75×10^6	235×10^2
8	109×10^5	297×10^2
9	86×10^4	49×10^5
10	65×10^6	115×10^4
11	105×10^5	185×10^3
12	81×10^6	110×10^4
13	75×10^6	85×10^4
14	90×10^5	100×10^5
15	60×10^7	145×10^4
16	45×10^6	87×10^4
17	208×10^4	95×10^5
18	95×10^5	45×10^4
19	110×10^6	125×10^4
20	50×10^7	112×10^4
21	68×10^5	250×10^2
22	297×10^3	150×10^3

TABLE - I Contd.

Sl. No. of samples	Standard plate count per ml	
	Pooled raw milk of Milk Supply Scheme, Patna	Pasteurized milk just before bottling of Milk Supply Scheme, Patna
23	190×10^4	115×10^4
24	42×10^7	102×10^4
25	175×10^4	210×10^2
26	150×10^5	152×10^3
27	252×10^3	198×10^2
28	35×10^7	182×10^2
29	259×10^3	265×10^3
30	31×10^7	62×10^5
31	205×10^4	185×10^2
32	195×10^4	45×10^5
33	m78×10^5	30×10^5
34	35×10^7	32×10^5
35	48×10^7	61×10^5
36	38×10^7	35×10^5
37	$32.x\ 10^7$	32×10^5
38	175×10^4	65×10^5
39	65×10^6	124×10^3
40	170×10^5	55×10^5
Minimum.	252000	18200
Maximum.	600000000	10000000
Average.	184000000	274000

TABLE - II

Micro-organisms recovered and their frequency distribution in milk samples

Sl. No.	Micro-organis	Pooled raw milk sample of Milk Supply Scheme, Patna		Pasteurized milk samples obtained before bottling of Milk Supply Scheme, Patna	
		Total samples processed 40		Total samples processed 40	
		Total samples positive for	Percentage of positive samples	Total samples positive for	Percentage of positive samples
1	Staphylococcus aureus	6	15	-	-
2	Staphylococcus epidermidis	13	32.5	9	22.5
3	Micrococcus roseus	2	5	2	5
4	Micrococcus luteus	2	5	-	-
5	Micrococcus (sp.)	3	7.5	2	5
6	Streptococcus faecalis	2	5	-	-
7	Streptococcus lactis	1	2.5	-	-
8	Streptococcus faecium var durans	-	-	1	2.5
9	Bacillus subtilis	11	27.5	6	15
10	Bacillus cereus	7	17.5	-	-
11	Bacillus alvei	2	5	2	5
12	Bacillus firmus	1	2.5	2	5
13	Bacillus lentus	1	2.5	1	2.5
14	Bacillus brevis	1	2.5	-	-
15	Bacillus coagulans	1	2.5	1	2.5
16	Bacillus megaterium	2	5	2	5
17	Escherichia coli	4	10	2	5
18	Alcaligenes faecalis	1	2.5	-	-
19	Pseudomonas aeruginosa	1	2.5	-	-
20	Providence B group	2	5	-	-
21	Citrobacter freundii	1	2.5	-	-
22	Aeromonas liquefaciens	9	22.5	-	-

TABLE - III

Physiological and bichemical properties of *Micrococcus* and *Staphylococcus* species.

Sl. No.	TESTS	SPECIES				
		Micrococcus luteus	*M.* (*sp.*)	*M. roseus*	*Staphylococcus aureus*	*S. epidermidis*
1	Catalase	+	+	+	+	+
2	Glucose	-	d	d	+	+
3	O-F test	-	O/-	O/-	F	F
4	V-P	-	-	-	+	d
5	Nitrate reduction	-	d	d	+	d
6	Pink pigment	-	-	+	-	-
7	Phosphatase	-	-	-	+	d
8	Coagulase	-	-	-	+	-

\+ = Positive for the test.
\- = Negative for the test.
d = Doubtful for the test.
O = Oxidative.
F = Fermentative.

TABLE - IV

Important physiological and biochemical characters of *Streptococcus* strains.

Sl. No.	Physiological and biochemical reactions.	SPECIES		
		Streptococcus Lactis	*Streptococcus faecalis*	*Streptococcus faecium var durans*
1	Catalase activity	-	-	-
2	Growth at 45°C	-	+	+
3	Growth in 6.5% NaCl	-	+	+
4	Aesculin hydrolysis	d	+	+
5	Hippurate hydrolysis	-	-	-
6	Gelatin liquefaction	-	-	-
7	Litmus milk	RAC	RAC	A
8	Glucose	+	+	+
9	Arabinose	-	-	-
10	Maltose	+	+	+
11	Trehalose	+	+	-
12	Salicin	-	+	-
13	Mannitol	+	+	-
14	Sorbitol	-	+	-
15	Sucrose	+	+	-
16	Lactose	+	+	+
17	Haemolysis			

\+ = Positive for the test.

\- = Negative for the test.

d = Doubtful for the test.

R = Reduction, A = Acid, C = Clot.

TABLE - V

Important physiological and biochemical characters of Bacillus species isolated from milk samples.

Sl. No.	Physiological and biochemical reactions.	SPECIES							
		Bacillus cereus	B. subtilis	B. megaterium	B. lentus	B. firmus	B. brevis	B. alvei	B. coagulans
1	Gram's reaction	+	+	+	+	v	v	v	+
2	Motility	d	+	+	+	+	+	+	+
3	Spore	+	+	+	+	+	+	+	+
4	Citrate utilization.	+	+	+	-	-	d	-	-
5	Gelatin hydrolysis.	+	+	+	-	+	+	+	-
6	Glucose	+	+	+	-	-	+	+	+
7	Arabinose	-	+	+	-	-	-	-	d
8	Mannitol	-	+	+	-	-	+	-	d
9	Indol	-	-	-	-	-	-	+	-
10	V-P	+	+	-	-	-	-	+	d
11	Nitrate reduction	+	+	-	-	+	d	-	d
12	Urease	d	d	d	+	-	-	-	-

v = Variable; generally positive in young cultures.
+ = Positive for the test.
- = Negative for the test.
d = Doubtful for the test.

TABLE - VI

Important physiological and biochemical characters of *Alcaligenes faecalis* isolated from milk sample.

Sl. No.	T E S T S	*Alcaligenes faecalis*
1	Motility	+
2	Oxidase activity	+
3	Citrate utilization	-
4	Growth on MacConkey	+
5	Gelatin hydrolysis	-
6	Nitrate reduction	-
7	H_2S production	+
8	Urease	-
9	Indole	-
10	Glucose	+
11	Maltose	-
12	Lactose	-

\+ = Positive for the test.

\- = Negative for the test.

TABLE - VII

Important physiological and biochemical characters of *Escherichia coli*, *Citrobacter freundii* and *Providence B group*.

Sl. No.	TESTS	SPECIES		
		Escherichia coli	*Citrobacter freundii*	*Providence B group*
1	Indole production	+	-	+
2	M.R. reaction	+	+	-
3.	V-P reaction	-	-	-
4	Citrate utilization	-	+	+
5	Glucose	AG	AG	-
6	Lactose	AG	AG	-
7	Arabinose	AG	AG	-
8	Sucrose	-	AG	-
9	Adonitol	-	-	-
10	Dulcitol	-	AG	-
11	Mannitol	AG	AG	-
12	Inositol	-	-	A
13	H_2S production	-	+	-
14	Urease activity	-	-	-
15	Haemolysis	-	-	-
16	Gelatin hydrolysis	-	-	-

A = Acid, AG = Acid and gas.
\+ = Positive for the test.
\- = Negative for the test.

TABLE - VIII

Important physiological and biochemical characters of Aeromonas liquefaciens and Pseudomonas aeruginosa.

Sl. No.	Physiological and biochemical reactions.	Aeromonas liquefaciens	Pseudomonas aeruginosa
1	Motility	+	+
2	Oxidase activity	+	+
3	Growth at 37°C	+	+
4	Citrate utilization	+	+
5	Growth on MacConkey	+	+
6	Gelatin hydrolysis	+	+
7	Glucose (gas)	+	-
8	Lactose (acid)	+	+
9	Sucrose (acid)	+	+
10	Mannitol (acid)	+	-
11	Sorbitol (acid)	+	+
12	Indol production	+	-
13	Nitrate reduction	+	+
14	V-P reaction	+	-
15	Urease	-	+
16	Haemolysis	+	+
17	Pigment	-	$+^{--}$

+ = Positive for the test.

- = Negative for the test.

$+^{--}$ = Green pigment in King's agar A medium.

DISCUSSION

DISCUSSION

Bacteriological quality of pooled raw milk samples obtained from Milk Supply Scheme, Patna :

In the present investigation forty pooled raw milk samples were collected from Milk Supply Scheme, Patna and were processed for total viable counts and isolation and identification of micro-organism. The bacterial count ranged from 2.52 lacs to 600 millions per ml with an average of 18.4 millions per ml.

Indian Standard Institute (1962) suggested certain standards for grading of raw milk. A standard plate count below 2,00,000 per ml was graded as very good milk, between 2,00,000 and 10,00,000 per ml as good milk, between 10,00,000 and 50,00,000 per ml as fair in quality and milk containing bacteria over 50,00,000 per ml was taken as poor in quality.

The total viable counts of three milk samples varied between 2,00,000 to 10,00,000 per ml and eleven milk samples varied between 10,00,000 to 50,00,000 per ml. The total viable counts for remaining samples were over 50,00,000 per ml.

Banerjee and Sen (1946), during their studies on bacterial content of milk of the Calcutta Milk Supply Scheme, suggested an average bacterial content to be 22 millions per ml.

Here in this investigation the average bacterial count is 18.4 millions per ml and it is more or less in agreement

as reported by Banerjee and Sen.

Verma et al. (1944) examined collected milk samples from adjoining villages of Banglore city for bacteriological quality and reported 1 to 90 millions per ml.

In present findings altogether twenty seven samples had the bacterial count in between 1 to 90 millions per ml as reported by Verma et al.

Venkataswami et al. (1963) suggested a total count of one million per ml for a satisfactory quality of raw milk.

Three samples showed bacterial count lower than one million.

Lavania (1969) graded milk supply of Baraut town (Merrut) on the basis of bacteriological and chemical quality and reported the total viable count to be 1.76 millions.

Four samples of the present investigation resembled with the total viable counts reported by Lavania.

Kumawat et al. (1972) reported 2.9 lacs to 120 millions per ml in milk samples obtained from rural collection centres.

Twenty nine samples of the present investigation were found to be varied between 2.9 lacs to 120 millions per ml and thus it is in agreement with Kumawat et al.

Jain and Saraswat (1968) and Vijai and Saraswat (1968) reported plate counts of 6 millions to 11 millions per ml in milk drawn from rural collection centres.

Ten samples of the present investigation are in agreement with that reported by Jain and Saraswat.

Patro (1973) studied twenty raw milk samples of the Milk Supply Scheme, Patna and reported the total viable count to be 3.5 millions to 3,000 millions per ml.

Twenty six samples of the present investigation varied in between the range reported by Patro.

Laxminarayan and Iya (1955) and Kumawat et al. (1972) considered time factors as an important cause for high bacterial count in milk.

Bacteriological quality of pasteurized milk :

Forty pasteurized milk samples were processed for total viable counts. The total viable counts varied from 18.2 thousands to 10 millions per ml. The average being 2.74 millions per ml.

Banerjee and Sen (1946) reported that pasteurized milk samples collected at Calcutta gave plate counts varying from 3,75,000 to 33 millions. Some samples of the present investigation were in between the range reported by Banerjee and Sen.

Sen and Laxminarayan (1948) recorded an average total viable count of 1,32,000 in pasteurized milk collected from milk booths of Banglore city. The total viable count of eight samples in the present investigation were in the confirmity with the range reported by Sen and Laxminarayan.

Kalkbrenner (1949) reported a total viable count of pasteurized milk reaching a large town to be 40,000 to 7,00,000 per ml. In the present investigation seventeen samples showed lower total viable count per ml and other samples were above the range reported by Kalkbrenner.

Storegards (1955) reported that the total viable count of pasteurized milk of the Skane district of Sweden varied from 100 to 4,42,000 per ml.

The total viable count of nine samples agrees with the range reported by Storegards.

Vijai and Saraswat (1968) reported 32,000 bacteria per ml. In the present studies two samples resembled with the report of Vijai and Saraswat.

Ninan Thomas and Laxminarayan (1972) reported a total viable count of 2.7 lacs to 2.8 millions in pasteurized milk.

The average total viable count of the present investigation is 2.74 millions per ml, which is more or less in confirmation with the range reported by Ninan Thomas and Laxminarayan.

Patro (1973) reported the total viable count of pasteurized milk to be 3 lacs to 30 millions per ml. In some of the samples the author agrees with the findings of Patro (1973).

Microflora in pooled raw milk samples of Milk Supply Scheme, Patna :

During the course of present study the bacteria from forty pooled raw milk samples of Milk Supply Scheme, Patna were isolated and identified on the basis of morphological, cultural and bio-chemical characters.

Staphylococci :

Nineteen strains of Staphylococci were isolated from the pooled raw milk samples. Out of which six strains were coagulase positive and thirteen strains were coagulase negative. The coagulase positive strains were treated as Staphylococcus aureus and the coagulase negative strains were treated as Staphylococcus epidermidis.

Recovery of S. aureus strains from raw milk samples in the present investigation are in confirmity as reported by Clark and Nelson (1961), Nilson and Segerfeldt (1964), Worseek et al. (1960), Galton (1960) and Patro (1973).

From the Table II it is evident that coagulase positive Staphylococci were distributed in 15 per cent of pooled raw milk samples.

The percentage incidence of S. aureus in the pooled raw milk of Milk Supply Scheme, Patna is in confirmity with those reported by Krishna Mohan and Misra (1967) from the same milk.

The presence of S. aureus in a significant number

in pooled raw milk samples warrants public health attention. S. epidermidis is non-pathogenic to man.

Micrococci :

About 67.77 per cent of the total bacterial population of milk was reported to be Micrococci by Laxminarayan and Iya (1955).

The present investigation is not in confirmity with the above authors because Micrococci constituted only 17.5 per cent in present studies.

Ranganathan et al. (1964), in summarising the species of Micrococcus recorded 13 species of Micrococcus including Micrococcus luteus and Micrococcus reseus. In the present findings only three species of Micrococcus were isolated and they are Micrococcus luteus, Micrococcus roseus, Micrococcus (sp.).

Streptococci :

Only two strains of Streptococci were recovered during the present course of investigation and they were Streptococcus faecalis and Streptococcus lactis. These species have also been described by Ranganathan et al. (1964) from raw milk samples.

Patro (1973) also investigated the above species of Micrococi in pooled raw milk samples of Milk Supply Scheme, Patna.

Streptococcus lactis is non-pathogenic to man and usually in habits raw milk. The presence of Streptococcus

faecalis in raw milk samples indicates contamination of milk from faecal sources. Certain strains of Str. faecalis have been reported to cause mild outbreaks of food poisoning and urinary infections either independently or mixed with E.coli (Wilson and Miles, 1955, Breed et al., 1957).

Aerobic spore forming bacteria :

The aerobic spore formers encountered in the pooled raw milk samples of present investigation are Bacillus subtilis, Bacillus cereus, Bacillus alvei, Bacillus firmus, Bacillus lentus, Bacillus brevis, Bacillus coagulans and Bacillus megaterium.

Varma et al. (1950) studied the incidence of spore forming aerobes in market milk in the country and encountered B. subtilis, B. cereus, B. megaterium, B. lentus, B.coagulans, B. brevis, B. sphericus and B. stearothermophilus.

The Bacilli species isolated in the present investigation is in agreement with Varma et al. (1950) and Patro(1973).

The occurrence of B. cereus in the pooled raw milk samples has been recorded for the first time in Bihar. This strain is pathogenic to man. The presence of B. cereus in significant number in pooled raw milk samples also warrants public health attention. The other strains of Bacilli are non-pathogenic to man. They are widely distributed in the soil, water, dust and spoiled food products and the milk is easily contaminated with the Bacillus strains from these sources. Improperly cleaned and unsterilized utensils used at

various stages of production and handling also constitute an important source for these organisms.

Escherichia :

During the course of bacteriological analysis Escherichia coli was isolated from 10 per cent of pooled raw milk samples.

Gopal Krishna and Laxminarayan (1949), Gopal Krishna and Iya (1951), Seshiah et al. (1965), Venkataswami et al. (1963), Patro (1973) have reported E. coli from raw milk. E. coli strains isolated from pooled raw milk samples in the present study confirms with the findings of the above mentioned workers.

Alcaligenes :

Only one sample was positive for Alcaligens faecalis which constituted only 2.5 per cent of the milk samples.

Sharma et al. (1967) reported A. faecalis as the co-aetiological agent in dysenteric disorders from Calcutta. Patro (1973) also reported that A. faecalis was distributed in 15 per cent of samples obtained from local suppliers and 5 per cent of farm raw milk.

Pseudomonas :

Pseudomonas aeruginosa was distributed in 2.5 per cent of pooled raw milk samples.

Isolation of Pseudomonas aeruginosa from pooled raw milk in this study is in confirmation with that reported by Thorne and Nilson (1962) and Patro (1973).

Providence group :

Providence B. group of organisms were present in two milk samples which constituted 5 per cent of total bacterial population.

Omprakash et al. (1966), Prema Bhat et al. (1971) and Patro (1973) have reported the presence of Providence B group of organisms in raw milk samples.

The presence of Providence B group of organisms in milk is perhaps due to unhygienic production.

Citrobacter :

Citrobacter freundii was isolated from the pooled raw milk sample of Milk Supply Scheme, Patna which is in conformation with the findings of Patro (1973).

Aeromonas :

Aeromonas liquefaciens investigated in the milk samples is in confirmation with the Patro (1973).

Microflora in pasteurized milk:

Staphylococci :

During the course of present investigation it was

observed that only Staphylococcus epidermidis comprises 22.5 per cent of total bacterial population in pasteurized milk. This strain of Staphylococcus is non-pathogenic to man. Patro (1973) reported the incidence of S. epidermidis to be 60 per cent in pasteurized milk and S. aureus to be 30 per cent.

But here in this investigation the present of S. aureus was not detected in the pasteurized milk.

Micrococci :

During the course of present study it was observed that Micrococcus roseus and Micrococcus (sp.) were distributed in 5 per cent of the samples.

Murray (1952) isolated Micrococci as dominant one in freshly pasteurized milk.

Shroff and Bhat (1955) also isolated Micrococci from the pasteurized milk collected from the distributing centres in Bombay. This investigation supports the idea of Murray, Shroff and Bhat.

Streptococci :

Streptococcus lactis and Streptococcus faecium var durans were isolated from the pasteurized milk samples.

Slanetz (1938) reported sixty strains of Streptococci in pasteurized milk.

Alexander and Higginbottom (1953), Ninan Thomas and Laxminarayan (1972) and Patro (1973) also reported the presence

of above species in pasteurized milk.

The absence of Str. faecium var durans in the pooled raw milk samples and its presence in pasteurized milk indicates post pasteurization contamination and unhygienic handling of milk.

Aerobic spore formers :

The aerobic spore forming bacteria encountered in the present studies are Bacillus megaterium, B. subtilis, B. alvei, B. coagulans, B. lentus and B. firmus.

Galsloot (1953) noticed that B. subtilis was present in abundance in immediately pasteurized milk.

Murray (1952), Shroff and Bhat (1955) and Patro (1973) reported spore bearing bacteria from pasteurized milk. The spore forming bacteria isolated from pasteurized milk samples are in agreement with the findings of above workers.

Escherichia :

Escherichia coli constituted only 5 per cent of the total bacterial population in pasteurized milk samples.

Alexander and Higginbottom (1953), Seshia et al. (1965) and Patro (1973) reported the presence of E. coli from pasteurized milk. E. coli strains isolated from pasteurized milk samples are thus in coincidence with the reports of above workers.

Alexander and Higginbottom (1953) and Venkataswami et al. (1963) have reported heat resistant strains of E. coli

from milk. It is quite possible that certain strains of E. coli present in pooled raw milk survived pasteurization. The presence of coliform bacteria in pasteurized milk may be due to under pasteurization, adding raw milk to vat, during the period of pasteurization, rinsing equipment with contaminated water, exposure of milk and milk equipments to dust and fly contamination, hand contamination of equipments and in adequate cleaning and sanitation as stated by Haskell (1952).

In view of the inadequate sanitory conditions existing in the pasteurization plants in India, the author agrees with the views of Warner (1953), who recommended boiling of pasteurized milk before consumption, inspite of any detrimental effect it may have on the nutritive values of the milk. This procedure will eliminate the risk of any public health hazard due to the consumption of contaminated pasteurized milk.

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S U M M R Y

S U M M A R Y

The objective of the present study was to determine the bacteriological quality and to investigate the bacterial flora of the raw and pasteurized milk of the Milk Supply Scheme, Patna.

Forty raw and forty pasteurized milk samples obtained from the pasteurization plant before bottling were examined. The total viable count was determined with the help of standard plate count technique. The total viable counts were 2.52 lacs to 600 millions per ml for raw milk and 18.2 thousands to 10 millions per ml for pasteurized milk. The average total viable count for raw and pasteurized milk was 18.4 millions and 2.74 lacs respectively.

The bacteriological analysis of raw milk samples resulted in the recovery of Staphylococcus aureus, Staphylococcus epidermidis, Micrococcus luteus, Micrococcus roseus, Micrococcus (sp.), Streptococcus faecalis, Streptococcus lactis, Bacillus subtilis, Bacillus cereus, Bacillus alvei, Bacillus firmus, Bacillus lentus, Bacillus brevis, Bacillus coagulans, Bacillus megaterium, Escherichia coli, Alcaligenes faecalis, Pseudomonas aeruginosa, Providence B group, Citrobacter freundii and Aromonas liquefaciens.

Whereas the bacteriological analysis of pasteurized milk samples resulted in the reocovery of Staphylococcus epidermidis, Micrococcus roseus, Micrococcus (sp.), Streptococcus lactis, Streptococcus faecium var durans, Bacillus megaterium,

Bacillus subtilis, Bacillus alvei, Bacillus coagulans, Bacillus lentus, Bacillus firmus and Escherichia coli.

The presence of Bacillus cereus was recorded from the raw milk for the first time in Bihar.

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