INDICATOR ORGANISMS AS INDICES OF SANITARY QUALITY FOR MILK AND SOME MILK PRODUCTS.

THESIS PRESENTED

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كليه الطب البيـــــــــــطري

قسم الرقابه الصحيه علي الاغذيـــه

قـــــــرار

لجنه الحـــــــكم والمناقشـــه

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Introduction

INTRODUCTION

The presence of microorganisms in food is not necessarily an indicator of hazard to the consumer or of inferior quality. Except for a few sterilized products, every mouthful of food contains some innocuous yeasts, molds, bacteria, or other microflora.

Most foods become potentially hazardous to the consumer only when the principles of hygiene and good manufacturing practice (GMP) are violated. If food has been subjected to conditions that could allow entry and/or growth of infectious or toxigenic agents, it may become the vehicle for transmission of disease such as summer diarrhea or staphylococcal food-poisoning. The detection of the causative agents or marker organisms of the objectionable contaminations is necessary for the prevention of such hazards.

As milk is a very suitable medium for the growth of many pathogens, and because raw milk as well as cheese made from raw milk such as Damietta and Kareish cheese, are subjected to contamination, either directly or indirectly, from different sources including producing animal, milk producers and handlers, occasional outbreaks of diseases traceable to dairy products do still occur in spite of advances in dairy manufacturing processes. Whenever man has led a nomadic life, accompanied by herds of domestic animals, he has made extensive use of milk, or its products, for food. As the fermented milk (yoghurt) beverages form a very interesting link between primitive

and modern dairying, it has a beneficial effect and is often prescribed by physicians for persons suffering from intestinal disturbances, so the examination of this product is very important for the previously mentioned microorganisms.

Therefore, this work was planned to study the value of some indicator organisms as indices of sanitary quality and of hazards to the consumer of milk, cheese (Damietta and Kareish) and yoghurt (Zabady) in Behera Governorate, and to evaluate the control measures adopted during production and handling of milk and the previously mentioned milk products.

LITERATURE REVIEW

In this review, the indicator organisms explained in detail as indices of sanitary quality for milk and some milk products such as cheese and yoghurt.

I. Coliform organisms:

NEWMAN (1951) examined the rather profused literature on the occurrence and detection of Bacterium coli in the milk and concluded that the presence of these organisms in milk can not in itself be regarded as an index to the hygienic quality of the milk.

PIRAUX, et al. (1952) examined samples of freshly delivered milk, milk at different stages of processing, and milk after overnight storage from 19 pasteurizing plants and found that the milk delivered at the plants was in general of poor quality and coliform counts was > 25.00 O/ml. Also they added that post-pasteurization contamination of the milk was common and the coliform count increased 10-fold or more after pasteurization in 43% of milks.

LITSKY, et al. (1953a) stated that the coliform group conventionally employed as indicators of pollution may consist of a large number of species of non faecal origin. The numbers of coliform group may persist in water and soil for long period of time and therefore might not be indicative of recent pollution. It was also demonstrated that these organisms may multiply in a soil or water enviroment.

LYONS and MALLMANN (1954) examined 131 Cottage cheese samples obtained from 8 dairies and found that about 84 (64%) samples contained coliforms and the pH of about 84% of samples ranged from 5.0 to 5.6. Also they reported that large percentages of all Cottage cheese samples collected contained coliforms in amounts of 220 or more organisms/100gm. and nearly all samples of bulk packaged Cottage cheese were heavily contaminated with coliform organisms.

SADEK and HASSAN (1955) held the view that the concentration of salt added to milk as well as the atmospheric temperature may affect formation of gas holes in Damietta cheese caused by coliforms. The author found that the addition of 5% salt at a temperature of 11 °C and 11% salt at 28 °C was sufficient to eliminate gas holes in cheese.

SADEK and EISSA (1956) examined 100 samples of market Damietta cheese, grouped into 4 grades on the basis of their coliform content. Defects in the flavour and texture were dependant upon the extent of coliform contamination. Also they found relationship between incidence of coliforms, salt content and acidity, a salt content of 9.5% or an acidity of 1.2% exerting an inhibitory effect. Acidity varied from 0.12 - 1.5%.

EL-SADEK and HAMED (1957) examined 100 samples of market milk collected in Cairo, 14 were found to be boiled, and found that coliforms were present in all samples and were found in 54.7% of the raw samples up to the 10^6

dilution and in 42.5% of the boiled samples up to the 10^2 dilution.

KERELUK and GUNDERSON (1959) studied the effect of lower temperature storage on the growth of E. coli and A. aerogenes (-6 F) and found that the coliforms decreased but the A. aerogenes were more resistant to the lower temperature storage than E. coli.

TUCKEY (1959) stated that outbreaks of gastroenteritis caused by coliform bacteria are rare.

MURRAY (1960) examined 7.432 samples of pasteurized (H.T.S.T.) milk which were taken direct from the plant, bottles in distribution, cans for hotels and hospitals and on 11.400 washed bottles and found that the proportion of milk samples containing coli-aerogenes bacteria varied from 12.9 to 27.8% (incubated at 37 °C) and 0.0 to 6.2% (incubated at 44 °C), and the % being highest during the summer months. He added also that of the 165 strains of E. coli isolated none was a pathogenic serotype, similarly no pathogenic serotype was found in the bottles. Finally he concluded that the plant management was good, no coli-aerogenes bacteria were found in the bottles.

McCOY (1961) stated that in the examination of foods, the presence of intestinal inhabitants should be taken to indicate a lack of cleanliness, not safety. He asserts that the safety of foods can be assessed only by examining for the presence of pathogens.

NIKHINSON, et al. (1962) examined a total of 375 samples of pasteurized milk and dairy products produced in a Kharkov dairy for pathogenic coliform bacteria and found that the pathogenic serotypes O_{55}, O_{26}, and 408 were detected in 3 samples of milk, 1 of kefir, 1 of smetana, 5 of quarq and 5 of quarq products. They also reported that 2 of the workers engaged in quarq manufacture were found to be carriers of pathogenic serotypes.

GHONEIM (1963) examined market Damietta cheese samples and found that coliforms were present in 70% of examined samples. The highest titre was 10^{-7}, with a mean of $10^{-4} \pm 0.14$ 10. He could isolated E. coli, Staph. aureus and Str. faecalis from 25%, 13.5% and 4% of the samples respectively.

SHIFRIN and OSATILOUSKAYA (1963) reported an outbreak of light food poisoning due to E. coli type O_{26}.

MOURSY and NASR (1964) examined 40 Kareish cheese samples and found that the coliforms were present in all examined samples at variable titers ranging from 10^{-3} to 10^{-8}, with a mean value of 10^{-6}. Faecal coli proved to exist in 95% of examined samples. They also could be isolated Staph. aureus from 2 samples (5%).

REINNEKE (1965) isolated 1.193 colonies from 129 soft cheese samples and reported that 393 were identified and belonged to the genus Escherichia, 564 to genus Aerobacter and 236 were classified as other coliforms. It was showed that the A. aerogenes were strong gas formers, causing the

majority of the blowing defects, while the genus Escherichia preferred an acid medium, the genus Aerobacter formed a smaller proportion of the total coliform microflora in high-fat soft cheese than in low fat varieties.

BOTTAZZI (1966) examined 35, 12, 12, 12, and 12 raw milk samples which were obtained from producers, by poorly maintained milking machine, with properly maintained equipment, by milking machine and shedcooled and hand-milked respectively and found that the mean levels coliforms/ml. were 15.387, 2.238, 400, 140.176 and 33.222 respectively.

KIELWEIN (1966) examined 2.279 milk samples collected from Südwürttemberg-Hohenzollern district and found that of 2.132 samples 22.1% had a colititer of 10^{-5} or greater. In only 25 of 68 farms using milking machines was the machinery properly cleaned.

HALL, et al. (1967) examined 490 food specimens for numbers and types of coliform organisms, including the EEC. They compared plate counts in VRB to counts obtained by isolation by enrichment in LST Broth and found that the LST Broth method produced a higher % of isolations. Also they determined the presence of E.coli by use of EC Medium incubated at 44.5 $\pm$ 0.1 C and found only 40.4% of the positive EC tubes, however contained E. coli. Only 0.6% of the specimens were contained EEC.

HUSKEY (1967) examined bacteria isolated from Cottage soft cheese and found that all isolates were Gram negative

lactose fermenters primarily A. aerogenes were 37.5%.

SAKAZAKI, et al. (1967) noticed that EEC cause diarrhoea not only in children but in adults as well.

DEVOYOD, et al. (1968) examined 2 batches of Roquefort cheese samples made by the traditional method during the manufacture and ripening and found that coliform counts of $10^5 - 10^6$. During 10 days after renneting they found that coliforms decreased. Also they added that coliform counts remained low (< 10/gm.) by 16 days after renneting (6 days after salting).

EL-SADEK, et al. (1968) examined market samples of skim-milk soft cheese (Kareish) for the incidence of coliforms and found that the majority of samples were contaminated with such organisms to a various extent. According to the presumptive coliform test, examined samples could be classified into the following three classes:

Class A: 15 samples (18.75%) in which coliforms were absent in 0.1 gm. of cheese.

Class B: 18 samples (22.5%) in which coliforms were absent in 0.001 gm. but could be detected in 0.1 gm. or 0.01 gm. of cheese.

Class C: 47 samples (58.75%) in which coliforms were present in 0.0001 gm. of cheese.

HUDEC (1968) examined 427 samples of yoghurt bacteriologically and found that 67.77% had coliform counts complying with the standard (max. 200/gm), including 54.4%

with counts of 10/gm., 27 samples (6.3%) had counts of 20000 - 30000/gm.

BRAG and KAMPE (1969) examined 53 French camembert cheese samples, 15 Danish cheese and 16 Swedish cheese samples, all purchased in Sweden, for incidence of pathogenic bacteria. They found that 62.3% of French samples, 40% of Danish and 93.8% of Swedish had E. coli counts of < 100/gm., while 24.3, 13.3 and 75% had coliform counts of < 1000/gm. respectively.

INSALATA, et al. (1969) examined 5.719 industrially processed food samples for the incidence of coliforms and E. coli and found that 8.5% and 0.3% of the samples were contained coliforms and E. coli respectively. They also found that 4.8% of the samples contained both coliforms and enterococci.

BODILY, et al. (1970) mentioned that certain serological groups of E. coli were known to produce severe diarrhea in infants and young childern.

CERAN (1971) reported that the initial coliform count in laboratory-prepared yoghurt gradually decreased. The reductions were 65% after the 1st hr., 93.5% after 2 hr., and 98.65% after 3 hr., of incubation at 44 - 45 °C, and 99.97% after 1 hr., and 99.99% after 24 hrs. of storage at 5 °C. The mean values for initial milk acidity and yoghurt acidity after 1, 2, 3, and 4 hrs. incubation and 5 and 24 hrs. storage were 8.1, 17.9, 22.1, 29.5, 34.8, 39.0 and 48.8 °S. H. respectively. Coliform survived in

23.3% of yoghurt samples, the higher the acidity of the yoghurt the greater was the reduction in coliforms. He also suggested that an antibacterial factor in addition to yoghurt acidity reduced the coliform count.

GORBACH, et al. (1971) found that certain strains of E. coli produced an enterotoxin which cause diarrheal disease in human.

JONES (1971) examined 1052 milk samples, 586 were being pasteurised and 466 were untreated. He could be isolated 5 and 122 presumptive E. coli type I strains from pasteurised and untreated samples of milk respectively.

MATSIEVSKII, et al. (1971) observed an acute outbreak of food poisoning caused by E. coli O_{124} among 198 children and 90 personnel members (including a milker) of a childern's sanatorium. It was found that the same strain isolated from unrefrigerated milk from cows kept on adjoining land and tended by sanatorium personal.

ABDEL-RAHMAN (1972) examined 60 samples of Egyptian soft cheese and found that coliform organisms, E. coli fecal type could be detected in 55% and 15% of market Damietta cheese samples, in 20% and 15% of tinned Damietta cheese samples and in 90% and 75% of Kareish cheese samples respectively.

ABO-ELNAGA (1972) stated the effect of sodium chloride (7%) on the growth of coliforms in raw milk which initially contains 10^6 coliforms/ml. He found that the coliforms

increased slightly during the 1st 2 days, then decreased to approximatly 10/ml. after 7 days.

KUMAWAT, et al. (1972) examined 220 milk samples collected from rural collection centres (121) and City markets (99) and found that the coliform count $<$1000/ml. in 40 of 121 milk samples and in 15 of 99 milk samples. They considered that a count of $<$1000 coliforms/ml. was reasonable from market mild solded under good conditions.

TZANETAKIS (1972) found that 101 of 217 samples of yoghurt collected from Hessaloniki market contained coliforms, 55 at levels of $>$ 10/ml. The highest % of positive samples occured in Summer and Autumn. The positive samples had PH of 3.5 - 4.2. He also recorded the types that occured most frequently were E. coli I (35 samples), A. aerogenes I (18), irrigular I (12), intermediat I (9) and intermediat II (6), A. aerogenes II and 8 other irrigular types were each found in upto 4 samples. E. coli I occured most frequently in Summer, irrigular I in Autumn, A. aerogenes in Autumn and Winter, and other irrigular and irrigular and intermediate types in Winter and Spring.

HOLZAPFEL and MOSTERT (1973) analysed 26 cheese samples collected from 20 different cheese factories microbiologically for coliforms & enterococci and found that there was no correlation was found between the quality of the cheese and the number of coliforms and enterococci.

KALINA, et al. (1973) determined counts of E. coli at

the Ostankino dairy on 15 occasions during Feb-June on (i) raw milk, (ii) pasteurized, (iii) bottled milk during production and (iv) bottled milk after 24 hrs. at room temperature and found that the E. coli counts in raw milk was 10^5/ml., while in pasteurized milk no E. coli. They noticed that the Bottling caused an increase in E. coli. Finally they considered that E. coli could serve together with Str. faecalis as an indicator of pasteurized efficiency.

PAPAVASSILIOU, et al. (1973) examined 100 commercial yoghurt samples collected from Athens area and found that the only species found in 30 of 100 samples were E. coli in 24, E. coli type II in 4, Klebsiella in 3, Cloaca in 1 and an atypical strain in one samples.

PARK, et al. (1973) examined Camembert cheese made from pasteurized milk inoculated to contain, approximately 100 cells of EEC/ml. They enumerated E. coli at intervals during the manufacture and ripening of the cheese using MPN technique and found that the growth of E. coli was minimal until after curd was cut and hopped but rapid growth ensued and populations in excess of 10^4/gm. appeared in some cheeses 5 hrs. after the cheese making process began. Also they found that the numbers of viable E. coli were decline after overnight storage with a drop in pH value of this cheese to 5.0 or below. From other hand they reported that salting of cheese and 1 day of ripening at 15.6 °C caused a further decline in the numbers of viable E. coli and this decline continued during the rest of the

week at 15.6 °C and during storage at 10 °C (50 F). They concluded finally 0 to 9 weeks at 10 °C were required before cheese was free of viable E. coli.

BROOKS (1974) examined 12 pasteurized and 20 raw milk samples and found that no coliforms were detected in pasteurized milk but in all 20 raw samples coliforms were detected. He also found that 16 of 20 raw milk samples contained E. coli. He also examined 111 samples of Queensland chedder cheese and reported that the coliforms and E. coli were present in 109 and 60 samples respectively. The author mentioned that E. coli was cosidered to be more indicative of direct faecal contamination than coliform.

FAHMY and YOUSSEF (1974b) examined market Damietta cheese samples and found that the average coliform count was 93748 organisms/gm., 42.2% were E.coli 44 °C (+), and 6.75% were intermediate E. coli type 2. They also reported that the level of salt and acidity in the 30 samples ranged from 6.019 - 15.128% and 0.20 - 1.92%, respectively. The concluded that the high salt and acidity concen. were associated with low coliform counts.

FANTASIA, et al. (1974) examined over 2.000 cheese samples and found approximately 10% of them containing E. coli belonging to serogroups associated with diarrheal diseases, the majority of these isolates were detected shortly after the initial food outbreak and belonged to the following serogroups: $O_{124} : B_{17}$ (lactose negative); $O_{124} : B_{17}$ (lactose positive); $O_{112} : B_{124}$; $O_{124} : B_{15}$; $O_{128} : B_{12}$; and $O_{127} : B_{8}$.

MANOLKIDIS, et al. (1974) examined 35 samples of Telemea cheese and found that only 5 were free from coliforms, but the majority of samples contained 10 coliforms/gm. E.coli I was found in 51.68% of surface samples and in 59.39% of samples taken from the interior of the cheese. They also reported the other strains which were found in surface samples as Irregular I, Irregular A, Intermediate I & II and A. aerogenes I & II.

PARK, et al. (1974) examined 48 French cheese samples which was implicated in an outbreak of food borne infection due to EEC $O_{124} : B_{17}$ in U.S.A., and could be isolated serotypes $O_{18} : B_{20}$; $O_{128} : B_{12}$ and $O_{125} : B_{15}$, while the serotypes $O_{124} : B_{17}$ failed to detection.

SINGH and RANGANATHAN (1974) examined 285 samples of milk and milk products comprising 50, 30, 78, 27, 10, 5, 10, 5, 15 and 25 samples each of cow raw milk, cow pasteurized milk, buffalo raw milk, buffalo pasterurized milk, raw cream, pasteurized cream, salted butter, unsalted butter, Cheddar and Processed cheeses and ice-cream respectively. They found that in all a total of over 1200 coliform colonies were isolated only 167 isolates representing one from each samples of milk and milk products were found to give positive reactions to the IMViC, EIJKMAN and other tests and such isolates were taxonomically identified as strains of E. coli. All the 167 isolates of E. coli were categorised in 4 groups on the basis of biochemical tests and motility as 113 isolates were E. coli I (Grade I),

34 were E. coli I (Grade II), 9 were E. coli II (Grade III) and 11 were E. coli III (Grade IV). Also they found out of 147 E. coli- I biotype cultures, 40 were serologically positive for enteropathogenic E. coli (EEC) groups and hence considered important from the public health point of view.

DOMETT (1975) collected 100 coliform isolates from cheese and found that they belonged to Escherichia, Enterobacter and Klebsiella.

MOUSTAFA, et al. (1975) examined 64 market milk samples collected from street vendors and shops in Assiut city and found that 22% contained E. coli.

SACK (1975) stated that the enterotoxigenic E. coli are known to produce diarrheal disease in human by colonizing the anterior region of the small intestine and there producing a heat-labile (LT) and/or a heat-stable (ST) enterotoxine that induces fluid secretion.

IKONOMOV, et al. (1976) examined 109 samples of White pickled cheese and found that no coliforms were detected in 99.1% of the samples, while the remaining samples contained 10 - 100 coliform organisms/gm.

MEHLMAN, et al. (1976) estimated that an "Infectious dose" of 10^8 to 10^9 viable cells (E. coli) must be consumed in a contaminated food products to cause illness.

NIELSEN (1976) stated that outbreaks of gastroenteritis caused by coliform bacteria are rare.

COLLINS-THOMPSON, et al. (1977) found in a survey of Canadian cheese that 13.6% of semisoft and 18.1% of soft cheeses contained more than 1600 coliforms/gm. Also, the numbers of faecal coliforms exceeded 1600/gm. in 0.8% of semisoft and 2.1% of soft cheeses. Although this dose not necessarily indicate a level of contamination capable of causing illness if EEC were present, it indicates the occurrence of unnecessary coliform contamination during the manufacture of these cheese varieties. They also concluded that the disease-causing potential of large numbers of E. coli in dairy products should not be ignored. Finally they reported the proposed standards per gram as follows: Total coliforms m = 500, M = 1500, fecal coliforms m = 100, M = 500, for cheeses made from pasteurized milk, but total coliforms m = 5000, M = 50.000, fecal coliforms m = 500, M = 1000, for cheeses made from unpasteurized milk. The level of acceptable contamination denoted by "m" and the level of organisms considered entirely unacceptable denoted by "M".

EL-BASSIONY (1977) examined 100 fresh Kareish cheese samples collected from Assiut market and found that the incidence of the enteric group E. coli was 67%. He also proved that Kareish cheese had been produced and handled under neglected hygienic conditions.

FRANK and MARTH (1977) analyzed 106 soft and semisoft cheese samples including Camembert, Brie, Brick, Muenster and Colby cheeses for faecal coliforms and serotypes of

EEC, and found that 57.5% were contained less than 100 fecal coliforms/gm. and 17.0% contained over 10.000 fecal coliforms/gm. Also they reported that serotypes of EEC were notdetected in any of these samples.

FRANK, et al. (1977) stated that relatively high levels of coliform contamination would be necessary to produce cheese likely to cause EEC food borne illness.

PAPABASSILEIOU and OIKONOMOU. STAMATELOPOULOU (1977) examined 50 pasteurized milk samples and found that the coliform count ranged between 2 and 16000/100ml. with 16 samples contained $<$50/ml. Among coliforms, E. coli I and II were the most common strains. They also added that the results indicated some improvement in the bacteriological quality of pasteurized milk in Athens but point out the need for further improvement.

TZANETAKES, et al. (1977) found that the coli-aerogenes bacteria were detected in 15,16 and 17 out of 20 Manouri, 19 Anthotyros and 20 Myzithra cheese samples respectively. The MPN exceeded 10/gm. in all 15 Manouri samples, all 17 Myzithra and in 14 of the 16 Anthotyros samples where the bacteria were detected. They also reported that 15 out of 58 strains isolated were identified as E. coli which were found only in Anthotyros and Myzithra cheese. The sodium chloride content were 1.36, 1.07 and 0.79% respectively.

MARTINEZ and FERNANDEZ (1978) examined samples from 11 ripened Serena cheese and found that a high variation in the coliform count which was 0.9 - 90.5 $\times$ 10^2/gm. in 10

samples and 1.3 x 10^7/gm. in the remaining samples.

OTTOGALLI, et al. (1979) examined 16 samples of goat's milk cheese, 19 of Mozzarella and 24 of Crescenza cheese and found that the coliform count were 10, 10^3 and 10^3/gm. respectively. They also analysed Crescenza cheese during processing and ripening and reported that the number of coliform/gm. was 10^4 in raw milk but zero after pasteurization. On the other hand they added that the number was 10^5 in the cheese curd, and 10^4 during ripening.

PATTERSON and JACKSON (1979) found that E. coli incubated at 1 or 4 °C became increasingly sensitive to Violet Red Bile Agar. They explained that the increased sensitivity was most marked with exponential phase cultures.

SHELAIH (1979) examined 105 Egyptian soft cheese samples (70 of Damietta and 35 of Kareish cheeses) collected from different localities in Cairo and Giza and found that coliform organisms were present in market Damietta, canned Damietta and Kareish cheese in 82.86, 60 and 94.29% of the samples respectively. The mean coliform counts (MPN) were 87.82 x 10^5 ± 81.19 x 10^5, 103.25 x 10^2 ± 60.03 x 10^2 and 74.4 x 10^4 ± 31.65 x 10^7 respectively. He could be isolated E. coli from 2.86% to 34.29%. Serological identification of isolated E. coli revealed identification of serotypes O_{78} : K_8: B_-, O_{86}: K_{61}: B_7 and O_{127} : B_8.

VARABIOFF (1979) examined 870 yoghurt samples collected from 11 factories in Brisbane and found that 42% of the samples exceeding the recommended level of 10/gm., the % of

the samples which complied with requirments for coliforms and E. coli type I were 97 and 99 respectively. He also considered that storage life of yoghurt should be at least 3 weeks under refrigeration.

ALEKSIEVA (1980) examined 33 cow's milk white pickled, 65 ewe's milk white pickled cheeses, 21 and 29 samples respectively of corresponding brines for incidence of coliforms and found that the coliform contents was low, only 3 of the 98 cheese samples showing contents of 10 - 100/gm., and no coliforms were detected in 0.1gm. of the remaining 95 samples. He also added that the coliforms were represented by E. coli, E. aerogenes and Citrobacter intermedius.

BURZYNSKA (1980) examined 175 production runs of homogenized fresh cheese samples collected from 26 dairy factories and found that > 30% of finished cheese samples contained coliforms and E. coli at titers of 1 - 10000 million/gm. It was concluded that homogenized fresh cheeses present a serious health danger, in particular childern.

GLATZ and BRUDVIC (1980) found that three enterotoxigenic strains and one non enterotoxigenic strain of E. coli were grown in milk at an initial pH of 6.5 to 8.5. One strain produced detectable levels of heat-labile enterotoxin in all cultures with an initial pH above 6.5. Also they tested 78 commerical cheese samples for the presence of E. coli and found that none of the 136 E. coli isolates obtained produced either heat-labile or stable enterotoxin, as measured in standard assays.

KIELWEIN (1980) examined 151 fermented products samples and revealed that many were contaminated with enterobacteria. He also found that the enterobacteria were found more often and in greater numbers in Quarq than liquid products (cultured milk, yoghurt, kefir and koumiss).

FEDER (1981) examined 564 of acid curd cheese samples collected from 12 cheese factories in lower Saxony and 1043 of curd samples from 14 dairies and found that no coliform bacteria in 42.9% of the cheese samples. He also reported that 16.8% had $> 5 \times 10^3$ coliform bacteria/gm. and no coliform bacteria in 83.1% of the curd samples, but only 1.8% had $>5 \times 10^3$ coliforms/gm.

LUCK and DUNKELD (1981) examined 130 samples of 4 - 8 weeks old cheese collected from 10 factories of South Africa and found that fecal E. coli were present in 86%. They also reported that the counts of fecal E. coli and total coliform ranged from <1 to 50000/gm., while 43% of the samples were contained <100/gm. and <1 to 36×10^6/gm., while 40% were contained <100/gm., respectively. They also found that the correlated factor between total coliform and faecal coliform was 0.75, while between total coliform and faecal streptococci was 0.31. Out of 540 isolated enterobacteriacea colonies 46% were E. coli.

TESONE, et al. (1981) examined 142 cheese samples and found that the total coliform counts were $\leq 10^2$, $10^2 - 10^3$ and $>10^3$/gm. in 62, 63 and 17 samples respectively. The fecal coliform counts were $<10^2$, 100 - 500 and $>$ 500/gm. in

106, 21 and 15 samples respectively.

COOKE, et al. (1982) compared a total of 158 colonies characteristic of E. coli which were isolated during routine product quality surveillance from 38 milk powder, 34 protein products, 48 butter and 35 cheese samples by using Enterotube, Microbact and Minitek methods with the Conventional techniques described in Edwards and Ewing (1972) and they found that the Enterotube significantly better than either Microbact or Minitek when they copared with Edwards and Ewing (1972).

JOHNSTON, et al. (1982) examined 243, 72 and 683 raw milk samples collected from farms in the West of Scotland from housed day & night, housed at night and outdoors day & night respectively. They found that the % of the samples from which coliforms isolated were 93.0, 83.3 and 92.0 respectively but the % of the samples from which E. coli isolated were 52.7, 36.1 and 37.5 respectively. Also they added that the % of the samples from which antibiotic resistant E. coli isolated were 23.5, 16.7 and 5.4 respectively. At the end they concluded that the incidence of both E. coli and antibiotic resistant E. coli in milk was higher when the cattle were housed day & night than when they were outdoors.

STORPER, et al. (1982) reported that after 2 weeks storage of bovine milk samples at - 18 °C the viability of isolates of E. coli was reduced by 9.7% and a further reduction in viability, ranging between 5 and 20% was recorded

following storage for anther 2 weeks.

WOOD, et al. (1983) examined 5, 5, 5, 11 and 12 food samples consumption patterns of U.S. students temporarily living in Guadalajara which collected from the Supermarket, Cafeteria, Street vendors, Restaurants and Houston restaurants respectively for the incidence of bacterial enteropathogens in foods from Mexico and found that the mean (MPN/gm) for coliforms, fecal coliforms were 3.349, 1.108, 844, 828 and 561, 529, 940, 232, 487 and 189 respectively. Also they reported that the enterotoxigenic % were 40, 0, 100,27 and 17% respectively. From other hand they examined 7, 8, 11, 9, 8 and 9 food samples which prepared in private Mexican homes and reported that the mean count/gm. for coliforms and faecal coliforms were 3×10^{5}, 3×10^{4}, 8×10^{5}, 2×10^{5}, 1×10^{3} and 8×10^{4}, 1×10^{2}, 1×10^{4}, 2×10^{4}, 2×10^{3}, 5×10^{2} and 8×10^{4} respectively. Finally they found that the food obtained from Mexican homes showed generally higher counts of coliforms and fecal coliforms than those obtained from commercial sources in Mexico, Houston and the foods in Mexico, both from homes and commercial sources, commonly contained E. coli and occasionally enterotoxigenic E. coli but foods in Houston were not contaminated with E. coli or enterotoxigenic.

BRODSKY (1984) analyzed 250 freshly formed Cheddar cheese samples (120 made from pasteurized, 62 from raw, 39 from sub-pasteurization heat treatment and 29 from undesignated treated milk) and found that the coliforms were detected in

61 (25.8%) and fecal coliforms confirmed in 46 (19.5%) of the 236 samples tested, with geometric mean counts/gm. of 133 and 136 respectively. He also reported that the incidence of coliforms was highest in Cheddar cheeses made from pasteurized milk (29.7%) compared with the incidence of raw milk cheese (22.0%) and cheese made from milk which had been subjected to sub-pasteurization heat-treatment (28.2%), but conversely, the incidence of fecal coliforms was lowest in pasteurized milk cheese (17.8%), followed by raw milk cheese (22.0%) and highest in heat treated milk cheese (25.6%). Also he examined 127 60-days aged cheese samples and found that the coliforms were detected in 37 (31.2%) and fecal coliforms confirmed in 22 (18.3%) samples, with geometric mean counts/gm. of 92.5 and 79.3 respectively. Finally he concluded that these results suggested that producers of Cheddar cheese should had no difficulty in meeting the microbiological standards adopted by the Health Protection Branch, Health and Welfare Canada.

NELSON, et al. (1984) examined 120 raw milk samples for enumeration of coliform organisms by three methods Petrifilm VRB, VRB agar and MPN with Lauryl sulphate tryptose and Brilliant green lactose bile broth (LST and BGLB) and found that there was a significant difference between the three coliform enumeration methods. Also they added that the MPN method produced higher coliform estimates than either Petrifilm VRB or VRB agar.

CHUBB, et al. (1985) surveyed bacteriological quality

of milk which collected from 3 small goat herds and found that coliform level was 1×10^2 colony forming units/ml.

II. Enterococcus organisms:

OSTROLENK and HUNTER (1946) demonstrated that, in 37% of 51 faecal specimens examined, enterococci occured in equal or in greater numbers than E. coli. In the remaining 63% of the specimens, E. coli exceeded enterococci numerically by from one to five decimal dilutions. They also concluded that the lower number of enterococci, as compared with E. coli in human and animal faeces does not in itself necessarily minimize the potential sanitary significance of fecal Streptococci.

KOSIKOWSKY and DAHLBERG (1948) reported that Str. faecalis was able to grow and survive in Cheddar cheese in large numbers for a considerable time when the cheese was ripened at 50 or 60 F.

SKADHAUGE (1950) differetiated Str. faecalis from Str. faecium by showing that the former organism grows in a medium containing 0.04% potassium tellurite, whereas growth of the latter organism was inhibited.

HANNAY and NEWLAND (1951) examined 2 Roqueforts, 5 Danish Blues, 4 Stiltons and 5 Cheddar cheese samples and found that 8 samples contained haemolytic streptococci and the number present varying from 1×10^3 to 1.7×10^6/gm. Also they added that all haemolytic streptococci were isolated from 4 varieties of cheese samples divided into 2 groups,

Str. faecalis var. zymogenes and Str. durans.

SHARPE (1952) examined the faecal flora of 65 healthy infants of up to 1 month of age and found that the flora contained streptococci which were more frequent in bottle-fed infants and the majority of the streptococci belonged to group D.

LEININGER and McCLESKEY (1953) studied the total coliform, B. coli and enterococcal counts of surface water ranging from a drainage canal to the rivers and concluded that the enterococcal count was more reliable than the coliform test in differentiating between recently polluted and relatively clean water supplies.

REINBOLD, et al. (1953) studied a selective plating medium for the isolation and identification of enterococcus group of streptococci which based up on the ability of the enterococcus to utilize sodium citrate and reduced ditetr-azolium chloride and grow in the presence of 0.01% sodium azide.

MOORE (1955) summarized the first report as follows" in an outbreak ascribed to milk at a school in New York state, 78 of 127 children and 4 of 5 teachers were taken very ill with abdominal pain, nausea, drowsiness, vomiting and diarrhoea within 2 hrs. of drinking school milk which obtained from mastitic cow herd affected by non-hemolytic Streptococcus.

BARNES (1956a) found that the Str. faecalis and Str.

faecium had different reduction and fermentation reactions. He reported that under appropriate conditions, Str. faecalis reduced 2, 3, 5 - triphenyltetrazolium chloride strongly, while Str. faecium reduced this compound weakly or not at all.

DACK (1956) reported that the symptoms of Streptococcus food-poisoning tend to be vague and variable. The illness was described as being milder than Staph. food-poisoning but had been reported to involve nausea, sometimes vomiting, colicky pains and diarrhea. He added also that incubation periods as short as 2 hrs. and as long as 32 to 60 hrs. had been reported.

SOLBERG, et al. (1957) examined 11 samples of raw milk and 29 of different varieties cheese samples collected from different parts of Norway and found that the Str. faecalis was commonly present in Norwegian cheese and a high heat resistance exhibited by some of the strains indicated that they probably originated from the raw milk and had survived H.T.S.T. pasteurization. They could be isolated 5 strains of Str. faecalis and 3 of Str. liquefaciens from the milk samples and 18 strains of Str. faecalis and 2 of Str. liquefaciens from the cheese samples.

STOCKER (1958) examined 250 strains of acid-producing streptococci which were isolated from China-blue-lactose agar plates of pasteurized milk for heat-resistant and found that about 25% were destroyed by heating in milk at 74 °C for 40 sec., but about 72% of these strains survived

heating at 72 °C for 40 sec. Also he added that many strains survived a temperature of 74 °C with a holding-time of 38.5 sec., but very few cells survived 77 °C. On the other hand he reported that the optimum growth temperature of 80% of the strains was 42 °C and many coagulated milk in 24 hr. at this temperature. Finally he identified the strains as Str. faecium (80%) and these organisms were numerous in raw milk supplies and although 90% of them were killed by H.T.S.T. pasteurization at 72 °C, many survived in pasteurized milk, dried milk, cheese and other milk products.

KERELUK and GUNDERSON (1959 b) studied the effect of lower temperature (-6 F) storage on the growth of Str. faecalis and Str. faecalis var liquefaciens and found that the enterococci remained relatively constant and Str. faecalis var liquefaciens was more resistant to the lower temperature storage than Str. faecalis and E. coli.

BARTLEY and SLANETZ (1960) found that the type of streptococci present in faeces of human beings and animals indicated that it might be possible, in certain instances, to distinguish between human and animal faecal contamination, while typical Str. faecalis may not always be the predominant type of streptococcus in human faeces.' It was not found in the faeces of most domestic animals, thus its presence in water would indicate contamination of human origin.

RAJ, et al. (1960) stated that a high recoveries of enterococci as compared to the low numbers of coliforms obtained from the same samples of frozen sea foods are indirect evidence that the enterococci are better indicators of contamination in such foods.

HASHIMOTO (1961) studied the characteristics and incidence of enterococci in milk and milk products, meat and faeces and found that the enterococci were predominated in heat-treated milk products. Str. faecalis was commonly found in raw milk but Str. faecium was predominated in heat-treated foods. Also he reported that the strains found in dried milk were divided into 7 sub-types on the basis of carbohydrate fermentation and the enterococci had a higher longevity than coliform bacteria and survived for 15 - 17 months in dried milk held at room temperature.

DEIBEL and SILLIKER (1962) reported that a total of 23 enterococcus strains were fed to two and sometimes three human volunteers in an effort to elicit food-poisoning symptoms. Each culture was consumed after it was grown in whole sterile milk or on the surface of commercially sterile ham slices. Six strains of Str. faecalis var. liquefaciens were consumed after complete liquefaction of gelatin. In addition, strains of Str. faecalis were consumed after having been grown in media which altered the energy metabolism e.g arginine, gluconate, malate and pyruvate. They found that in no instance were any of the above conditions of growth conducive to the production of food-poisoning symptoms in

volunteers. Moreover, no evidence was found to indicate that either the age of the culture or the disruption of the cell was a factor in the production or release of a toxic principle. It would appear that until the enviromental conditions (if any) for food-poisoning were defined the evidence obtained suggests that the association of enterococci and food-poisoning was questionable.

SARASWAT, et al. (1962) stated that the Citrate Azide medium which modified by increasing the azide concentration have a high selectivity for enterococci and found that 408 colonial isolates from plates of raw milk, cheese and butter could be identified as enterococci.

BALDOVIN-AGAPI, et al. (1963) found that 77.7% of cheese samples contained Str. faecium and 9.9% contained Str. durans, while Str.faecalis was not present in any of the samples examined.

GHONEIM (1963) examined market Damietta cheese samples and found that Str.faecalis was found in 4% of the samples.

SARASWAT (1963) reported the frequent occurence of enterococci in young cheese. He also observed that there were decrease in enterococcus and total counts of young Cheddar cheese ripened at 3.3 °C.

HASHIMOTO, et al. (1964) identified 601 enterococcal strains isolated from dried and pasteurized milk samples

as 533 Str. faecium, 21 Str.faecalis, 10 Str.faecalis var.liquefaciens, 3 Str.durans and 34 remained unidentified. Of the 577 strains isolated from dried milk, 92.3% were Str.faecium. They also added that on the basis of 6 fermentation reactions the Str.faecium were divided into 9 types of which those fermenting sucrose, glycerol, mannitol and arabinose and not fermenting sorbitol and raffinose were the most frequent in dried milk samples. Finally they reported that no typical Str.faecium strain was isolated from the pasteurized milk samples.

WHITTENBURY (1965) stated that the established tests for differentiation between Str.faecalis and Str.faecium are reducing activity and potassium tellurite tolerance only.

CLARK and REINBOLD (1966) examined 41 commerical Cheddar cheese samples obtained from 10 Iowa cheese manufacturing plants and could be isolated, characterized and identified 1.117 microorganisms. A total of 578 isolates (51.7%) of the microflora, were enterococci, of which 159 (27%) were identified as Str.faecalis, 59 (10%) as Str. faecalis var.liquefaciens and 15 (3%) as Str.faecalis var. zymogenes. Computed numbers of enterococci ranged from 54×10^{3}/gm to 49×10^{6}/gm. in sample having an Agar plate count of 49×10^{6}/gm. Also they found that the cheese samples containing the highest agar plate count 100×10^{6}/gm., had a computed enterococcus count of 210×10^{5}/gm. On the

other hand they revealed that the enterococci decreased from an average computed count of 36×10^5/gm. in the seven-days-old samples to 10×10^5/gm. in the 3 months-old samples.

JICINSKA and PESEK (1967) examined monthly samples which were taken in a drying plant of (i) raw milk from tankers, (ii) pasteurized milk (85 °C, 25 sec.) from storage tanks (5 - 7 °C), (iii) milk pasteurized a second time (95 °C, 15 sec.) immediately before drying, and (iv) dried milk for incidence of enterococci and found that the content of enterococci (~75%) Str.faecalis, with Str. liquefaciens, atypical Str. faecalis and Str.lactis-like organisms) in (i) rose steadily from ~10000/ml. in Jan. to 50000/ml. in Aug. In (ii) the counts were max.,~50/ml, in April-Aug. Samples were taken directly from the pasteurizer had counts of 0 - 88/ml. but in (iii) no enterococci were detected. In (iv) counts ranged from 100 to 300/ml. reconstituted milk, with no seasonal trend. Finally they reported that the daily examination showed highest values to occur before the weekly cleaning of the drier.

HASHIMOTO (1968) examined dried milk samples collected from 6 dairy plants for incidence, number and fermentative types of enterococci in 3 series of experiments and found that the counts of enterococci were < 1000/gm. in 308 of 373 samples tested and the fermentative types varied in the 3 series, but the most commonly encountered was a subtype of Streptococcus faecalis which fermented sucrose,

mannitol, arabinose and (weakly) glycerol. Finally he concluded that the bacteriological quality of dairy products in Japan has not changed during the period between the first series of expt. in May 1962 and the third in May 1966.

BRAG and KAMPE (1969) examined 53 samples of French camembert, 15 Danish and 16 Swedish cheeses, all purchased in Sweden, for incidence of Str.faecalis. They found that Str.faecalis counts were < 100.000/gm. in 37.3%, 40% and 68.8% of cheeses samples respectively.

DUCHENNE, et al. (1969) isolated 20 strains of enterococci, 12 out of 25 fresh and 8 out of 10 sealded cheese samples. They could be identified the isolated strains as Str.faecalis (60%), Str.faecalis var.liquefaciens (20%) and Str.faecium (20%), but Str.faecalis var.haemolyticum and var.zymogenes could not be detected.

INSALATA, et al. (1969) examined 5.719 industrially processed food samples for the incidence of faecal Streptococci and found that 10.6% of the samples were contained enterococci. Only 4.8% of the samples contained both enterococci and coliforms. Also they reported that the results of these work indicated that the value of enterococci as indicators of fecal contamination may depend to a large degree on the type of product and/or process being examined but enterococci may serve as useful indicators of the sanitary history of a product and of the industrial process involved.

JANOSSY (1969) examined 445 milk and milk products samples and found that 186 (41.7%) samples were contained Str.faecalis. Also he reported that the incidence of such organisms in processed cheese samples was 32%, while in the hard and semi-hard cheese was 43.3%.

KALINA (1970) proposed that the Str.faecalis and Str. faecium be transferred to the genus Enterococcus of Thiercelin and Jouhoud. However, this proposal was not generally accepted, and the genus Enterococcus was not recognized in Bergey's Manual of Determinative Bacteriology, 8 th edition.

FACKLAM (1971) examined 262 strains of group D Streptococci which were isolated from human sources. 142 isolates from blood cultures were included, 96 of these were submitted as isolates from clinical cases of subacute bacterial endocarditis and found that 98 Str.faecalis, 29 Str.faecalis var. zymogenes, 44 Str.faecalis var.liquefaciens, 27 Str.faecium, 13 Str.durans, 44 Str.bovis and 7 unspeciated Str.bovis-like group D isolates were identified.

ABO-ELNAGA (1972) stated the effect of sodium chloride (about 7%) on the growth of enterococci in raw milk which initially contain 10^6 enterococci and found that 126 strains were isolated from 30 raw milk after 2 days of storage comprised 58 enterococci.

KUMAWAT, et al. (1972) examined 220 milk samples

collected from Rural collection centres (121) and City markets (99) and found that enterococci count was 1000/ml. in 10 of 121 and 25 of 99 milk samples. They considered that the enterococcus count gives indication of the hygiene conditions during production and good quality milk should not contain 1000 enterococci/ml.

KALINA, et al. (1973) determined counts of enterococci at the Ostankino dairy on 15 occasions during Feb-June on (i) raw milk, (ii) pasteurized, (iii) bottled milk during production and (iv) bottled milk after 24 hrs. at room temperature. They found that the enterococci counts in raw milk was 10^3, while in pasteurized milk, enterococci counts remained at the 10/ml. until April (except for 2 samples in March with 10^3/ml.), then increased to 10^3/ml. Bottling caused no increase in the enterococci. After storage of bottled milk, enterococci counts increased 10 - 100 fold. Of the 618 strains of Str.faecalis isolated, 400, 10, 56 and 152 were in raw milk, pasteurized milk, bottled milk during production and bottled milk after 24 hrs. at room temperature respectively. It is considered that Str. faecalis could serve together with E.coli as an indicator of pasteurized efficiency.

FREKOPPOVA and FREKOPP (1973) could be isolated Str. faecalis from Lump and Bryndza cheese as well as from its whey.

BROOKS (1974) examined 12 pasteurized and 20 raw milk

samples and found that the enterococci alone were detected in 4 of 12 past. milk, while in all 20 raw milk samples. He also examined 111 samples of Queensland cheddar cheese and reported that the enterococci was present in 82 samples. The author concluded that enterococci and E.coli were considered to be more indicative of direct faecal contamination than coliform and recomended the use of enterococci as indicator for detection of faecal contamination of cheese.

EFTHYMIOU, et al. (1974) studied the sensitivity of an Enterococcus Selective Differential Medium (ESD) in comparison with other media and found that the (ESD) supported the fastest rate of growth and the maximum size of colonies, counts on this medium were in most cases possible within 17 hrs. of incubation, whereas the other media required 24 to 48 hrs. They also added that the utility of ESD for rapid, presumptive identification of enterococci was confirmed by serological and biochemical testing of 2,3,5- Triphenyltetrazoliumchloride differentiated colonies isolated from 18 cheese samples.

MOUSTAFA, et al. (1975) examined 64 samples of market collected from street vendors and shops in Assiut city and found that 8% contained Str.faecalis.

MALESZEWSKI, et al. (1976) examined 955 samples of Twarog cheese and found that the haemolytic streptococci were found only in 7.3% of the low-fat factory cheeses.

AHMED (1877) examined 100 samples of different types of cheese (Damietta, 50, Kareish, 25 and Hard cheese, 25) collected from the local Assiut city markets and found that the average counts of enterococci in different cheese samples were 30.94 x 10, 126.04 x 10 and 377.16x10 respectively. He also reported that the incidence % of Str.faecalis was found to be 22, 14 and 12 respectively.

BRUM, et al. (1977) examined 30 commercial raw milk samples from Santa Maria after laboratory pasteurization and found that 6.66% of the samples were showed Gram-positive colonies of thermoduric Streptococci. They also identified the 2 isolates as Str.faecalis by tests including incubation on TTC agar.

EL-BASSIONY (1977) examined 100 fresh Kareish cheese samples collected from Assiut markets and found that the incidence of some pathogenic micro-organisms regarding the Str.faecalis was 52%. He also proved that Kareish cheese had been produced and handled under neglected hygienic conditions.

PAPABASSILEIOU and OIKONOMOU-STAMATELOPOULOU (1977) examined 50 samples of pasteurized milk and found that 26 samples contained Str.faecalis at 1 - 1000/ml., and he concluded that contamination with human faeces being suspected in some of these cases. The results indicated some improvement in the bacteriological quality of pasteurized milk in Athens but point out the need for further improvement.

AHMED and EL-BASSIONY (1978) examined 100 milk, soft cheese, processed cheese, table butter and youghurt samples (20 of each) and found that the average counts of enterococcus on ESD media were 64×10^2, 20.6×10^3, 18.9 x 10, 24.8×10^2 and 79.4×10^2 respectively. They also reported the incidence percentage of Str.faecalis which were 60,75, 55,100 and 25% out of 60, 75, 60, 100 and 55 positive samples % on ESD medium respectively.

CARRASCO, et al. (1978) examined 20 strains of Streptococcus which were isolated from raw milk samples obtained from dairies in the Santa Fé province of Argentina. On the basis of biochemical tests they were identified as Str.faecalis (4), Str.faecalis subsp. liquefaciens (3), Str.faecalis subsp.zymogenes (1), Str.faecium (6) and Str. faecium subsp. durans (5), one isolate was not identified. They added also that the Str. faecalis subsp.liquefaciens was considered a possible substitute for lactic streptococci in cheese starters.

DAMDINSUREN and GRUEV (1978) examined a Mongolian cheese made by coagulating milk with 5 - 10% yoghurt (Tarag) at 80 - 90 C, without rennet or salt. They could isolate 50 Streptococcal colonies, but 15 of these colonies were selected for species differentiation tests. They found that all 15 colonies gave positive results in Sherman's tests for identification of enterococci. Of 15 strains only 2 strains appeared to be Str.faecalis but the remaining 13 strains appeared to be Str.faecium subsp.durans.

MOSSEL, et al. (1978) established that Streptococci of Lancefield's group D cause systemic disease in man. The evidence that they can also cause febrile gastroenteritis is only circumstantial. They also stated that however, there is no doubt that profilic growth of group D Streptococci in foods may lead to the formation of clinically significant levels of pressor amines. They added also that group D Streptococci have a function as indicator and index organisms-categories that should be clearly distinguished.

ALEKSIEVA (1979) concluded that the enterococci are more suitable than E.coli as hygiene indicators for yoghurt, and the examination of yoghurt for coliforms should be carried out within 24 hrs. of production.

ALEKSIEVA (1979) examined 92 samples of ewe's milk commercial Kachkavol cheese and found that the enterococci counts ranged from < 100 to 1.9 millionen/gm. in 29.3% of the samples, but 33.7% of the samples contained > 100.000/ gm. He also reported that 67.1% of the 137 enterococci strains isolated were identified as Str.faecium subsp. durans and 32.8% were Str.faecium.

FACKLAM, et al. (1979) examined a total of 155 strains of B-hemolytic streptococci serologically by conventional techniques (Lancefield extraction and capillary precipitin testing) and by Latex agglutination (LA) and found that the agreement between them was 97% when the instructions

of the manufacture for the LA technique were followed, but this agreement was 99% when modified autoclave extracts were used as antigens in the LA procedure. Also they tested a total of 82 strains of non-B-hemolytic streptococci by the three procedures and found that the agreement between conventional techniques and both LA procedures was 76%. Of 13 strains of Str.bovin, 10 did not react with the LA group D reagent but were serogroup D by conventional techniques.

GÖRNER, et al. (1979) considered that the enterococci that were present in all product samples in considerable numbers and were completely destroyed by the heat treatment could be employed as a criterion of the level of sanitation in the manufacture of thermaized products.

OTTOGALLI, et al. (1979) examined 16 samples of goat's milk cheese, 19 of Mozzarella and 24 of Crescenza cheese and found that the enterococci counts were 10^4, 10^6 and 10^5/gm. respectively. They analysed Crescenza cheese during processing and ripening and found that the number of enterococci was 10^5 in raw milk but 10^3 after pasteurization and 10^5 in the cheese curd and 10^6 during ripening. They also concluded that the most faecal streptococci in the raw milk were destroyed during pasteurization but recontamination strains which were resistant to heat treatment persist a part of the final cheese microflora.

SHELAIH (1979) examined 105 Egyptian soft cheese samples

(70 of Damietta and 35 of Kareish cheese) collected from different localities in Cairo and Giza and found that the mean total enterococci counts/gm. of market Damietta, canned Damietta and Kareish cheese were $132.53 \times 10^3 \pm 74.59 \times 10^3$, $45.79 \times 10^3 \pm 12.89 \times 10^3$ and $212 \times 10^3 \pm 65.9 \times 10^3$ respectively. He also reported that Str.faecalis proved to be the most prevalent type of enterococci found in examined samples of cheese, as it could be detected in 89.5% of the samples.

USAJEWICZ and WYSZKOWSKA-ZABORNIAK (1979) examined 14 Solan cheese samples which were obtained from 3 polish dairy factories and 4 production batches and stated that the high incidence of contaminant microflora especially of enterococci is attributed to poor production hygiene and enterococci are considered better hygienic indicators in Solan cheese than coliform titers prescribed for this purpose.

ALEKSIEVA (1980) examined 33 cow's milk white pickled cheese, 65 ewe's milk white pickled cheese, 21 and 29 respectively of corresponding brines samples for the incidence of enterococci and found that 30.3% of cow's milk white pickled (CMWP) and 18.5% of ewe's milk white pickled (EMWP) cheese samples contained no enterococci in 0.01 gm., but in 12.1% of CMWP and 17% of EMWP the incidence of enterococci was 100.000 - 500.000/gm. He also added that there was no relationship between the incidence of enterococci and salt content, titratable acidity or pH of CMWP or EMWP, and no differences in levels of contamination

were found between CMWP and EMWP brines. He reported that the microflora of cheese and brines consisted predominantly (91.3%) of Str.faecium and Str.faecium subsp.durans.

BATISH, et al. (1980) considered that the presence of enterococci in milk and milk products as indicators of faecal pollution, spoilage organisms and food poisoning agents.

BISSONNETTE, et al. (1980) examined 105 thermonuclease-positive (TNase-positive) cheese samples comprising 13 types and found that 9 (8.6%) contained microorganisms other than staphylococci as the major contaminants in which 6 samples contained Bacillus spp.comprising three species and 3 contained mainly enterococci (Str.faecalis) which were proven to be TNase producers. They also added that unlike staphylococcal TNase, a greater part of non staphylococcal TNase remains in the cheese homogenate after extraction of the enzyme at pH 3.8 instead of pH 4.5.

DAVE, et al. (1980) examined 2 batches of cheese samples and recorded that the enterococci increased initially during ripening and recorded a maximum values of 880000 and 530000/gm. respectively, in the 2nd month, then decreased again during the next 5 months. 44 isolates were identified as 29 Str.faecium, 8 Str.bovis, 2 each of Str. liquefaciens, faecalis and faecium subsp.durans and 1 Str.equinus.

KIELWEIN (1980) examined 151 fermented milk products

samples and revealed that many were contaminated with enterococci and the average counts of enterococci in kefir and cultured milk were higher than those of enterobacteria but no as high as the enterococci counts commonly found in ripened cheese.

PARK, et al. (1980) surveyed a total of 1204 cultures comprising 16 genera for production of TNase in milk and found that cultures other than staphylococcus capable of TNase production were restricted to two genera (Streptococcus and Bacillus). 19% of 338 group D streptococci comprising 4 species (85% of which were Str.faecalis) and 17% of 60 streptococci belonging to other groups produced TNase. They also added that the amount of TNase produced by streptococci was significantly lower than that produced by coagulase-positive staphylococci and the pH profile of the streptococci TNase was similar to that of the staphylococcal TNase. Finally they reported that the nuclease produced by streptococci was less heat stable than the nuclease produced by coagulase-positive staphylococci, and 50% of the activity of the streptococcal nuclease was destroyed in 40 - 60 min. at 100 °C.

LITOPOULOU-TZANETAKE, et al. (1981) examined 60 samples of traditional Greek yoghurt from retail outlets in Thessaloniki and found that the enteric bacteria (Str.durans, Str.faecalis and Str.faecium) were present in 30% of the samples. They reported also that the counts of Str.faecalis and faecium inoculated into ewe's milk decreased only

slightly during 6 days storage after making the milk into yoghurt (pH 4.0).

RAMOS, et al. (1981) examined 4 batches of cheese made by experimentaly procedure with pasteurized sheep's milk as well as one control manufactured from raw milk and found that the enterococci reached 6.9×10^{6} cell/gm. after 4 months of ripening in the control but in experimental cheeses comprised between 10^{4} - 10^{5} cell/gm. at the 4th month of ripening. They concluded that these bacteria were not completely killed during pasteurization.

SEDOVA, et al. (1981) studied the enteropathogenicity of the enterococci having proteolytic characteristic (Str. faecalis var. liquefaciens and Str. faecalis var. zymogenes) experimentaly on isolated loops of the small intestine of the rat and rabbit and found that these strains possessed enteropathogenic properties but the degree of the pathogenicity varied between the strains.

BATISH, et al. (1982) screened 510 isolates of enterococci recovered from milk and milk products for deoxyribonuclease (DNase) production and found that of the 166 (32.5%) DNase-positive cultures, 29 (5.7%) produced TNase that resisted boiling for 15 min. Also they reported that although the incidence of thermolabile DNase-positive enterococci was 50.5% in Cheddar cheese, the majority of TNase-producing enterococci was recovered from dried milks and infant foods. On the other hand, they added that on the

basis of biochemical, physiological and serological tests, all DNase-producing enterococci were characterized as Str. faecalis var.faecalis (21), Str. faecalis var.zymogenes (9), Str.faecalis var.liquefaciens(90), Str.faecium (23) and Str. durans (16) and the predominant TNase-positive types was Str. faecalis var.faecalis followed by Str.faecalis var. zymogenes. Finally they reported that 6 strains of enterococci were found to be toxigenic when tested in ligated rabbit ileal loops, infant mice and rabbit skin.

BRAG (1982) studied the definitions of the enterococci and faecal streptococci, occurrence of and sources of contamination, methods and media for culture and isolation of enterococci, and the role of enterococci in food poisoning and found that of 2000 samples of various foods, 98 contained enterococci at counts $> 10^3$/gm., 13 had counts exceeding 10^5/gm. (the limit of acceptability). 7 were therefore considered unfit for human consumption solely on the basis of the enterococcus counts.

MALIK (1982) examined 200 strains of enterococci isolated from various food products and found that Str.zymogenes, faecium, faecalis, durans and Str.liquefaciens were identified as 74, 48, 44, 18 and 16 strains respectively. Also he added that 33, 20 and 7 samples positive of milk and dairy products, prepared foods and infants foods showed the highest contamination rates of enterococcal contamination.

MUCCHETTI, et al. (1982) examined a total of 54 samples

of 20 Italian cheeses and found that the enterococci ranged from 10 to 170 x 10^6 cfu/gm. 31 strains were isolated and identified as 18 Str.faecalis, 8 Str.faecium and 5 Str. faecalis subsp.liquefaciens.

PETERZ (1982) isolated 47 strains of enterococci from foods (Str.faecalis and Str.faecium) and 6 Laboratory strains were used in a study on confirmation methods such as growth at 10 and 45 °C, survival for 30 minuts at 60 °C, growth at a Nacl conc. of 6.5%, and fermentation characteristics and found that 96% of the strains studied were positive in the heat tolerance test, and 100% in the Nacl test. Also he reported that a confirmatory procedure involving the catalase test, Gram staining and growth in the presence of 6.5% Nacl was recommended.

FARROW, et al. (1983) stated that the Str.faecalis and Str.faecium are quite distinct from the majority of the species in the genus Streptococcus according to the recent nucleic acid studies.

SUAREZ, et al. (1983) identified 820 bacterial strains which were isolated from 3 batches of Mahon cheese from Menorca and found that the enterococci consisted of 166 strains of Str.faecium subsp.durans, 107 of Str.faecalis, 84 of Str.faecalis subsp.liquefaciens and 18 of Str.faecium.

APPELBAUM, et al. (1984) used the Rapid Strept system (API system S.A., Montalieu-Vercieu, France) without additional tests, in the identification of 209 streptococcus

strains included 36 group D and found that this system correctly identified to species level 100% of the group D strains. Also they added that the method provided excellent identification rates of groups A, B and D.

APPELBAUM, et al. (1984) used 2 commercial methods, the API 20 S system and the Gram-positive Identification Card without additional tests for the identification of 241 streptococcus strains included 36 group D strains and found that the API correctly identified to species level 86.1% of group D strains, but GPI correctly identified 97.2% of group D strains. On the other hand they added that API correctly identified 41.9% of the strains to species, with 41.1% good likelihood but low selectivity, 15.8% incorrect, and 1.2% not identified, but GPI correctly identified to species level 66.0% of the strains, with 8.7% correct preliminary identification, 20.8% incorrect, and 4.6% not identified. Finally they reported that API provided excellent identification of group A, B and Str. faecalis strains, but GPI provided accurately identification of group A, B and D strains.

BECKER, et al. (1984) surveyed strains of several genera of bacteria for production of TNase and found that 103 isolates of D-streptococci failed to produce the enzyme.

SCHLEIFER and BÄLZ (1984) stated that the results of deoxyribonucleic acid-deoxyribonucleic acid and deoxyribonucleic acid-ribosomal ribonucleic acid hybridization

studies demonstrated that the Str.faecalis and Str.faecium are distantly related to the non-enterococcal streptococci (Str.bovis and Str.equinus) of serological group D and to other streptococci. On the basis of their results they propose that the Str.faecalis and Str.faecium be transferred to the genus Enterococcus (ex Thiercelin and Jouhaud) nom. rev. as Enterococcus faecalis (Andrewes and Horder) comb. nov. and Enterococcus faecium (Orla-Jensen) comb.nov., respectively.

III. Staphylococcus organisms:

HAUGE (1951) reported that 12 out of 31 persons who had eaten a meal of mashed potatoes, sausages and beer, developed typical symptoms of staphylococcal food poisoning. He analysed the foods consumed and found that a large numbers of ∝ -and B-toxin-producing staphylococci together with group B streptococci in the mashed potatoes. On the other hand he added that the unpasteurized milk which obtained from 2 cows had been added to the potatoes during mashing and the mash had been stored for 6.5 hr. under conditions favourable for bacterial growth before it was served. A clinical and bacterial examination of the 2 cows whose milk had been used revealed that one was shedding ∝ -and B-toxin-producing staphylococci from one quarter and finally he concluded that the milk from these cows was the source of the infection.

CECCARELLI and FRANCIA (1952) examined composite milk

samples which were collected from 382 cows and found that only 2 were positive for enterotoxic staphylococci.

WARING (1952) reported an outbreak of 285 children at 6 schools in the Oxford area and found that the cause of the outbreak was traced to school milk which had been adequately pasteurized by the H.T.S.T process and contained staphylococcal enterotoxin type of food-poisoning.

CHARLES (1953) reported that the milk and milk products were the vehicles of infection in 13 out of 310 outbreaks (including family outbreaks) of food poisoning and added that the staphylococci was responsible for 5 of these outbreaks. Also he added that a series of 8 outbreaks of staphylococcal food poisoning in schools in various parts of the country, in which the vehicle of infection proved to be spraydried skim-milk supplied by one particular firm, and the Staph.aureus was isolated from batches of spray-dried milk. Finally he added that the toxin was formed in the milk before spray-drying.

TAYLOR (1954) reported that 3 closely-spaced outbreaks of vomiting and diarrhoea which occurred at a hospital and involved 45, 25 and 23 cases respectively among both patients and staff. He added also that the infection appeared to be the milk which was produced at the hospital farm and was consumed raw. Although a good standard of hygiene was found on the farm, 2 of the dairy men yielded coagulase-positive Staph.aureus.

THATCHER, et al. (1956) found that 45% of examined raw milk cheese samples and 13% of pasteurized milk cheese contained coagulase-positive Staph.aureus. Also they reported that cheese made in Canada sometimes contained large number of toxigenic Staphylococci. On the other hand they concluded that the presence of large numbers of Staphyloccocci in cheese must be due to either gross contamination or to incidental contamination, followed by growth either in the milk or cheese or in both.

LEGLER, et al. (1957) reported an outbreak of staphylococcal food-poisoning involved 64 persons due to consumption of Camembert cheese. The causative organisms (Staph. aureus) was found in large number in cheese samples and in the stools of some patients.

SMITH (1957) stated that large numbers of Staphylococci in raw milk are unlikely to occur unless milk from infected udder is withdrawn under hygienic conditions and kept at a fairly high temperature.

LEGLER, et al. (1958) reported an outbreak of food-poisoning affecting at least 46 persons and found that all persons had consumed Camembert cheese from the same source and S.aureus was found in large numbers in this cheese samples and also in the stools of some of the patients.

NEUMAN, et al. (1958) reported 22 cases of staphylococcal food-poisoning, 13 of which were transmitted via salted

cheese and 2 via milk and cream.

HENDRICKS, et al. (1959) reported that 200 cases of staphylococcal food intoxication resulted from eating natural American Cheddar cheese that was 4 to 8 months old. Also they added that the cheese was made from raw milk and coagulase-positive B-hemolytic S.aureus of similar phage type was isolated from the cheese and the milk of two of eight herds supplying milk to the cheese factory.

TAKAHASHI and JOHNS (1959) studied the factors affecting the growth of staphylococci in raw milk and Cheddar cheese and found that the milk with a relatively low standard plate count support an active growth at 32 °C., and a moderate growth at 22 °C., whereas, almost no growth took place, even at 32 °C., in milk with a high standard plate count. They added also that large numbers of staphylococci in milk would be most apt to occure when bacteriologically clean milk was held at a fairly high temperature.

THATCHER, et al. (1959) examined 224 samples of Canadian cheeses made from raw and pasteurized milk and found that staphylococci were widely distributed frequently in large numbers in cheese made from raw milk while in cheese made from pasteurized milk no staphylococci were present.

WILSON, et al. (1959) held the view that a minimum population of 50.000 coagulase-positive staphylococci/gm.

is required to produce sufficient toxin to cause food-poisoning.

ALLEN and STOVALL (1960) mentioned that outbreaks of food-poisoning in Wisronsin during 1958, were traced to Colby-type cheese. They found that out of 48 cheese samples examined, 22 contained staphylococci, out of which 17 samples contained coagulase-positive S.aureus.

HAUSLER, et al. (1960) examined one or more samples from each lot of the unused Cheddar cheese which was responsible for Iowa Institution food-poisoning outbreak and found that 87% of the cheese in storage contained B-hemolytic coagulase-positive S.aureus. They also found that one strain was tested for entorotoxin production caused vomiting and diarrhea when fed to kittens.

CLARK and NELSON (1961) examined 20 raw milk samples collected from Iowa producers using bulk milk tanks and found that all samples contained coagulase-positive staphylococci in initial numbers of 25 to 3.300/ml. Also they added that the staphylococci multiplied in naturally infected milk held at 10 °C for 7 days but not in milk held at 4 °C for 7 days and the maximum staphylococcus count obtained being 360.000/ml.

MICKELSEN, et al. (1961) analyzed 125 samples of cheese, representing 20 varieties for the presence of staphylococci and found that 76% of the samples contained staphylococci, 70.4% contained S.aureus and 7.2% contained potentially

pathogenic coagulase-positive S.aureus. They also added that the ratio of S.aureus contamination was not the same for all varieties and a relationship exists between the presence of coliform organisms and S.aureus in cheese.

SHARPE, et al. (1962) compared the Baird-Parker medium with other media for the isolation of staphylococci from raw milk and cheese and found that on Mannitol Salt Agar only 49.9% of the total number of colonies from all samples were coagulase-positive and on Phenolphthalein Phosphate Agar 54.9% of phosphatase positive colonies were coagulase-positive but with Baird-Parker medium 85.2% of presumptive coagulase positive staphylococci were proved to be so on testing.

CHONEIM (1963) examined market Damietta cheese samples and found that S.aureus were present in 13.5% of the samples.

MICKELSEN, et al.(1963) examined 24 samples of commercial Cottage cheese obtained during winter months and 42 samples obtained during summer months and found that the incidence of samples containing staphylococci, S.aureus and S.epidermidis was higher in summer than in winter. Also they reported that 35 (53%) samples contained S.epidermidis, while 9 (14%) samples contained S.aureus. They also added that no correlation between the pH and the counts on either medium (TG and S-110) or between the pH and percentage of coagulase-positive organisms could be determined.

DONNELLY, et al. (1964) examined 13 samples from several lots of Cheddar cheese incriminated in staphylococcal food-poisoning as well as 343 samples purchased over a 3-years period in retial markets quantitatively for coagulase-positive staphylococci and found that of the food-poisoning samples, 11 contained coagulase-positive staphylococci in numbers that ranged from 50 to several million/gm. Of the markets samples, 20% contained coagulase-positive staphylococci in concentration ranging from less than 50 to more than 200.000/gm.

EPSOM (1964) recorded outbreaks of staphylococcal food-poisoning involving 100 patients, occured in London hospitals due to consumption of Newzealand 2nd grade cheese samples contained approximatly 200 million/gm coagulase-positive staphylococci.

MOURSY and NASR (1964) examined 40 samples of Kareish cheese and they could be isolated S.aureus from 2 (5%) samples.

SMITH and BAIRD-PARKER (1964) found that the addition of 50 ug of sulphamethazine/ml. of medium suppressed the growth and swarming of proteus species.

CASMAN and BENNETT (1965) stated that the slide double-diffusion test is especially valuable for the detection of enterotoxin in foods. It is technically simple and requires very small amounts (0.02 to 0.03 ml.) of reagents. The lines of ppt.are easily identified through their coalescence

with the lines formed by reference toxins, a property which is valuable in the examination of food extracts which may produce lines of ppt.not caused by specific reaction with antibody.

RIVAS, et al. (1965) examined 524 samples of different types of cheese and found that 474 samples contained staph. species of which 170 were coagulase-positive.

SHARPE, et al. (1965) examined 910 raw milk cheese samples and found that 9% of samples contained 500.000 staphylococci/gm.

FARONE (1966) examined 261 cheese samples and found that 9 samples were contaminated with S.aureus in counts of 100-20.000/gm.

BROEKE (1967) found that Baird-Parker medium valuable in ecological studies on food incriminated in staphyloenterotoxicosis. 97.5% of the 522 strains of S.aureus tested, isolated from human and food origins, developed characteristically and quantitatively on Baird-Parker medium.

CASMAN, et al. (1967) found that the number of coagulase-positive staphylococci which produced enterotoxins had been 50% for strains isolated from clinical specimens and 96.2% for strains isolated from food products.

CHESBRO and AUBORN (1967) studied the relationship between the staphylococcal growth and nuclease production in foods under varying conditions of temperature,

aerobiosis and competition from other microorganisms and concluded that the nuclease was produced under any conditions that permit growth of S.aureus and it was found that 15 min. at 121 °C was required to reduce the nuclease activity in Slurries of contaminated ham below the level present in the unheated Slurry. Also they reported that the nuclease and enterotoxin A production were shown to vary in synchrony for the 234 (Casman) strains of S.aureus.

DONNELLY, et al. (1967) examined serologically coagulase-positive staphylococci from cheese for enterotoxigenicity. They found that 9 of 155 cultures from market cheese and 7 of 77 cultures from food-poisoning cheese produced enterotoxin A, and that none of the cultures produced detectable levels of enterotoxin B.

DEVOYOD, et al. (1968) examined 2 batches of Roquefort cheese samples made by the traditional method during manufacture and ripening and found that micrococci/staphylococci counts of $\sim 10^{5} - 10^{6}$. During 10 days after renneting they found that micrococci/staphylococci a slightly decreased. Also they added that the coagulase-positive staphylococci declined to < 10/gm. by 16 days after renneting (6 days after salting).

DONNELLY, et al. (1968) studied the production of enterotoxin A in milk by use of variables of milk quality, initial numbers of enterotoxigenic staphylococci, incubation temperature, and time and found that in both raw and pasteurized milks having a low total viable count, enterotoxin

was detected in minimal incubation time of 6 to 9 hr. at 35 °C, 9 to 12 hr. at 30 °C, 18 hr. at 25 °C, and 36 hr. at 20 °C, after inoculation with 10^6 S. aureus cells/ml. They also reported that when similar milks were inoculated with 10^4 S. aureus cells/ml, enterotoxin was detected in 12 hr. at 35 °C, 18 hr. at 30 °C, 24 to 36 hr. at 25 °C, and 48 to 96 hr. at 20 °C. In high-count raw milk, enterotoxin was detected only in samples inoculated with 10^6 S. aureus/ml and incubated at 35 °C but generally, a conc. of 5×10^7 S. aureus cells/ml of milk was reached before enterotoxin A was detected.

THATCHER and CLARK (1968) recorded that even as few as 50.000 organisms of S. aureus/gm. have been implicated in food-poisoning cases from relatively fresh food, while processed food in which a large population of staphylococci have been destroyed by heating may nevertheless cause food poisoning owing to survival of the heat resistant entero-toxins.

ZEHREN and ZEHREN (1968) reported that the degree of acidity at early stages of manufacture was more important for the prevention of staphylococci multiplication and enterotoxin production than was the pH value in the final cheese.

LACHICA, *et al.* (1969) studied the relationship between heat-stable DNase and coagulase production for the developing more rapid diagnostic and quantitative

procedures for distinguishing toxigenic and pathogenic staphylococci from closely related saprophytic organisms and found that of the coagulase-positive strains, 95% produced a heat-stable DNase and 10 of 41 coagulase-negative strains produced a heat-stable DNase, 9 of 10 strains also produced enterotoxin. They concluded that these strains probably represent mutants that have lost the ability to produce coagulase. They also added that among the enterotoxin-producing strains, 93% produced coagulase and 95% produced heat-stable DNase.

SOMM (1970) examined soft-Camembert cheese through post-pasteurization contamination with certain staphylococci and found that the growth of these organisms was favoured by low acidity of milk (e.g pH $>$ 4.8). Also he added that when 30% of water was added to milk inoculated with 3000 S.aureus/ml, the acidity development was reduced, the min. pH attained being 4.82, and the resulting cheese contained 32×10^6 S.aureus/gm., 21 days after manufacture. In cheese from similarly inoculated undiluted milk (min.pH attained = 4.6), these organisms died out.

IENISTEA, et al. (1971) found that 12 out of 45 samples of Telemea cheese contained coagulase-positive staphylococci and the count reached 2.4×10^7/gm. They also found no coagulase-positive staphylococci present in 5 samples of bottled, pasteurized milk.

LACHICA, et al. (1971) studied the metachromatic

agar-diffusion methods for detection staphylococcal nuclease activity and found that the three convenient agar-diffusion methods have been developed that enable detection of the nuclease of S.aureus at conc.as low as 0.005 ug/ml in agar and broth cultures. They also added that for most strains of S.aureus, the zone of nuclease activity was usually observed after 1 to 2 hrs. of incubation.

ORTH, et al. (1971) examined the sera of several animals for suitability in coagulase testing and found that the assay for coagulase-reacting factor (CRF) activities of the whole sera indicated the following relative conc. of CRF: human > pig > rabbit > horse > bovine > chicken, and lamb but the plasmin activities of the different sera, arranged from the strongest to the weakest, were as follows: rabbit > human > lamb > horse > bovine, chicken, and pig. Also they reported that the pig serum was superior to the other sera for use in the plate test for coagulase and heparinized pig plasma was more suitable than citrated pig.

SIMKOVICOVA and GILBERT (1971) found that 92% of staphylococcus strains which isolated from food products involved in food-poisoning in England and Wales were enterotoxigenic.

ABDEL-RAHMAN (1972) examined 60 samples of Egyptian soft cheese and found that 10% of market Damietta cheese only were contained S.aureus.

ABO-ELNAGA (1972) stated the effect of Nacl (about 7%) on the growth of staphylococci and micrococci in raw milk

which initially contain 10^6 staphylococci and micrococci and found that the staphylococci reached a maximum of 152 million/ml. after 2 days, but after 5 days and through out the remainder of the storage period, the level fluctuated between 10^5 and 10^6/ml. 126 strains isolated from 30 raw milk after 2 days of storage were contained 68 staphylococci & micrococci of which 53 were staphylococci, 45 being coagulase-positive.

GHONEIM (1972) examined buffaloe's milk yoghurt inoculated with broth cultures of S.aureus and stored at 30-32°C - 1 °C and 4 °C. He found that the S.aureus survived for 20, 50 and 29 days respectively. The minimum pH values recorded during storage at the three temperature were correspondingly 5.5, 7.0 and 6.0 in inoculated and 8.0, 7.0 and 7.0 in control samples respectively.

HEGAZI (1972) stated that soft cheese is among the dairy products mostly incriminated in case of food-poisoning in Egypt. He added that the main reason of this problem is that most of such cheese offered in the market are made under primitive conditions from raw milk.

LACHICA, et al. (1972) studied the three factors for the MAD assay of staphylococcal nuclease in BHI Broth and found that the diameter of the pink zone of hydrolysis was related to time and temperature of incubation and to nuclease conc. Also they added that conc. as low as 0.005ug/ml and as high as 2.0 ug/ml were conveniently determined after 3 hrs. at 37°C.

P.H.L.S (1972) examined 424 routine food samples and 233 food samples were experimentally inoculated in the laboratory with staphylococci for detection of coagulase-positive staphylococci and for the recovery of staphylococci from the samples respectively. It was found that coagulase-positive staphylococci were isolated from 46 (10.9%) of the 424 routine foods, while a high recovery rates of coagulase-positive staphylococci were obtained from 233 foods inoculated in the laboratory.

BARRY, et al. (1973) stated that either the coagulase or TNase test may be used for routine identification of S.aureus, providing that the inoculum is prepared by pre-incubation in a small volume of BHI, and the use of two tests for identification of S.aureus can be viewed as aqul-ity control measure which is unnecessary most of the time but very reassuring when difficulties are uncovered.

CORDS and TATINI (1973) evaluated the relationships between growth of S.aureus and production of DNase and enterotoxin A in cheese and found that there was aclose correlation between the S.aureus population and DNase content in which 88 and 85 in Cheddar, Colby and Brick cheese respectively. They also added that while the viable S.aureus population declined during aging, both DNase and enterotoxin A persisted for an extended time (3 years at 4.4 °C) in cheese of normal or inhibited starter.

HOLZAPFEL and MOSTERT (1973) analysed microbiologically 26 samples of cheese from 20 different cheese factories for

staphylococci and found that fairly large numbers of staphylococci ($>10^4$/gm) were detected in most of the cheese.

TATINI, et al. (1973) evaluated staphylococcal enterotoxin A production in Blue, Brick, Mozzarella and Swiss cheeses from milk inoculated with different initial S.aureus population and found that the enterotoxin was detected under certain conditions (type of lactic starter and the strain of S.aureus) in Brick and Swiss cheeses. They also added that it was not demonstrated even with higher S.aureus population (5×10^7 /gm) and a complete starter failure in Blue cheese. From other hand they reported that the minimal S.aureus population associated with presence of detectable enterotoxin was influenced by the enviromental conditions in cheese.

TATINI, et al. (1973) investigated the question at what level of Staph.aureus reached in cheese enterotoxin was detectable. They found for Cheddar cheese detectable amounts at 2.8×10^7 Staph. aureus/gm. For Colby cheese these number were 1.5×10^7 /gm., and for Brick cheese 8.0×10^6/gm., but for Swiss cheese these counts varied from $0.7 - 13.0 \times 10^7$/gm. cheese, dependent on the strain used. They also added in Blue cheese at 4.8×10^7/gm. enterotoxin could still not be detected and for Gouda cheese these data were not known

LENGAUER and STUMTNER (1974) examined 242 raw milk samples for staphylococci that were coagulase-positive

(CPS)or DNAase-positive (DPS) and found that the incidence varied from 10% in July to 100% in Jan., and averaged 37% for CPS and 43% for DPS. They also added that 17% of the samples contained $10^3 - 10^4$ CPS/ml. and 3% contained 10.3×10^4 CPS/ml. Finally they concluded that the coagulase and DNAase tests seemed to be had equal value for rotuine testing of raw milk as 91% of the staphylococci examined gave the same reaction, either positive or negative to both tests.

PAYNE and WOOD (1974) surveyed the production of staphylococcal enterotoxins A, B, C, D and E among 200 strains of Staph.aureus using a double-diffusion immunoprecipitation technique. Of the 200 strains of Staph.aureus, 125 produced one or more enterotoxins and the most strains produced only one type but others produced up to 3 enterotoxins simultaneously. They reported also that 83 strains (41.5%) were produced only a single enterotoxin, of these, 59 strains were produced enterotoxin A, 3 were produced B, 8 were produced C, 11 were produced D, only 2 were produced E but 42 strains (21%) were produced more than one enterotoxin, 15 strains were produced A & D, 6 were produced A & E, and a further 6 strains produced A, C and D. No other mixtures of enterotoxins occured in $>$ 4 strains. They also found that enterotoxin A was occured most frequently and enterotoxin B least frequently.

ROBBINS, et al. (1974) compared several methods for examining large numbers of staphylococcal strains for production of the known enterotoxins (A - E) and found that

overall, the sac-culture-production method was superior for all-around enterotoxin production and could be used as a secondary method for confirmation when questionable results were obtained by other methods.

SMOLKA, et al. (1974) studied the effect of pH level and Nacl conc., alone and in combination, on the enumeration of unstressed and heat-stressed cells of three strains of S.aureus and found that counts of unstressed cells diminished only slightly with increases in Nacl, whereas heat-stressed cells showed a marked sensitivity to Nacl conc. of 4% and above, regardless of the pH level. They also added that a maximum counts of unstressed cells could be obtained quite effectively in a medium containing 7.5% Nacl adjusted to a pH range of 7.0 through 8.5 maximum. Counts of heat-stressed cells could not be attained on media containing 5% or more Nacl and the pH of the medium plus sample should not be outside a range of 5.5 through 8.5, even with a Nacl conc., below 4%.

GHAZVINIAN, et al. (1975) examined 212 fresh unsalted Iranian White cheese samples collected from 36 Teheran retailers between Oct. 1972 and May 1973 for total counts and coagulase-positive staphylococci and found that the latter were found in 79 samples, 48 in the range 10000 - 100000/gm.

MAYER (1975) tested 297 strains of heat-stable DNase producing staphylococci isolated from the milk of mastitic

cows and 143 ones from human clinical specimens for enterotoxinogenicity and on correlation between enterotoxinogenicity and other criteria, e.g production of coagulase, DNase and hemolysins and found that 45 (15.1%) of the bovine and 17 (11.9%) of the human strains produced enterotoxin. He also reported that the distribution on the different serological types in bovine strains was 11 (3.7%), 10 (3.4%), 4 (1.3%), 15 (5.1%), 1(0.3%), 3(1%) and 1(0.3%) for type A, B, C, D, E, AD and BD respectively, but in human strains was 3(2.1%), 4(2.8%); 3(2.1%), 1(0.7%), 5(3.5%) and 1(0.7%) for type A,B,C,D,E and AD respectively. Finally he added that all but one enterotoxinogenic strain (type C) produced coagulase, whereas 10 bovine and 4 human non enterotoxinogenic strains were coagulase-negative but heat-stable DNase-positive.

MOUSTAFA, et al. (1975) examined 64 samples of market milk collected from street vendors and shops in Assiut city and found that 17% of the samples contained S.aureus.

RAYMAN, et al. (1975) examined 91 enterotoxigenic strains and 103 cultures of S.aureus isolated from foods and clinical material respectively for the production of coagulase and TNase and the ability to ferment glucose and mannitol and found that with the exception of 4 strains of food, a complete correlation among these properties was observed. On the other hand they suggested that the TNase test be performed on cultures with doubtful coagulase reaction.

SOMMERFELD and TERPLAN (1975) stated that the detection of enterotoxins in foods, preexamination of the food on presence of TNase, subsequently only in case of positive results microbiological examination (including tests on formation of coagulase and enterotoxins) as well as extraction of enterotoxins from food is recommended with the batch adsorption procedure and serological detection of the enterotoxins by Ouchterlony-test instead of counter current immuno-electrophoresis.

TATINI, et al. (1975) reported that the production of DNase paralleled growth of S.aureus during the 24 hr incubation at 37°C. DNase and enterotoxin D were detectable within 2 hr at a S.aureus population of 2×10^6, whereas enterotoxin A was detected after 4 hr at a higher population of S.aureus. They also added that the stage of growth of S.aureus did not have any significant influence on DNase production, approximately the same amount of DNase having been produced by 2, 5 or 10 hr cells during subsequent incubation (2 - 6 hr). On the other hand they found that the DNase was detectable in all the substrates (BHI and NFDM) within 3 - 4 hr and at S.aureus population of about 5×10^5/ml. Enterotoxin A or D was detected, only subsequent to the detection of DNase with S.aureus population of 5×10^6/ml except in Gouda cheese whey of 1×10^7/ml.

MALESZEWSKI, et al. (1976) examined 955 samples of Twarog cheese and found that 7.8% of 587 high-fat factory

cheeses, 6.6% of 202 low-fat factory cheeses and 9.9% of 306 farm cheeses contained coagulase-positive staphylococci.

EL-BASSIONY (1977) examined 100 fresh Kareish cheese samples collected from Assiut market and found that the incidence of S.aureus was 16% of the samples. He also proved that the Kareish cheese had been produced and handled under neglected hygienic conditions.

NISKANEN and KOIRANEN (1977) examined a total of 276 S.aureus strains isolated from routine sampling, food poisoning outbreaks, and mastitic milk for production of enterotoxin A, B, C, D and E and found that 80% of strains isolated during investigation of food poisoning outbreaks were toxin producers, as were 51% of strains from routine food sampling, and 41% from mastitic milk samples. They also reported that the commonest toxin found from all samples was A and of the food-poisoning strains, 69% produced A either alone or in conjunction with other toxins. Enterotoxin A production was found in 29% strains from routine sampling and 49% from mastitic milk. Of all the toxin-producing strains, 53% produced A, 38% °C and 39% D. They also reported that all strains produced TNase and all toxigenic strains produced coagulase.

PAPABASSILEIOU and OIKONOMOU-STAMATELOPOULOU (1977) examined 50 pasteurized milk samples and found that 9 samples contained 1 - 1000 coagulase-positive staphylococci/ml. They

concluded that the results indicate some improvement in the bacteriological quality of past.milk in Athens but point out the need for further improvement.

STADHOUDERS, et al. (1977) stated that the growth of Staph. aureus during the cheese manufacture is more abundant as the proportion of starter is decreased, and with increasing age of the starter culture used, but is scarcely affected by the cooling temperature. They also concluded for reducing the growth of Staph.aureus as much as possible by the addition of 1% of a 24-h-old starter.

BATISH, et al. (1978) reported that the thermostable DNase producing staphylococci isolated from raw milk, Cheddar cheese and baby food also produced enterotoxin A, whilst those isolated from dried milk produced enterotoxin A and/or B. They also added that the strains were coagulase-positive but DNase - negative were considered non enterotoxigenic.

MARTINEZ and FERNANDEZ (1978) examined samples from 11 ripened Serena cheese and found that staph.counts ranged from $1.01 - 18.9 \times 10^6$/gm.

PARK, et al. (1978) examined samples of food, naturally and artificially contaminated with S.aureus for enterotoxin and TNase and found that 70 out of 88 naturally contaminated food samples (80%) were positive for TNase, while 22 (25%) were positive for enterotoxin. All enterotoxin containing samples were positive for TNase, except 2 samples

of egg sandwich filling. The samples containing entero-toxins comprised 31.4% of the TNase-positive samples. They also added that with the exception of the sandwich fillings, TNase could be detected in nearly all foods with $> 10^6$ S.aureus cells/gm., and the detection rate was 88% in cheese. From other hand they reported that of the 100 artificially contaminated food samples, TNase was detected in 63 (63%) and enterotoxin in 53 (53%). Finally they concluded that the TNase production by S.aureus was obviously dependent on the type of food in which the organism was grown.

SVESHNIKOVA, et al. (1978) found that 236 out of 300 raw milk samples supplied to the cheese factories contained staphylococci. They added also that coagulase-positive staphylococci were detected in 151 samples, and 22 out of 45 dairy farm workers and 35 out of 95 cheese factory workers carried coagulase-positive staphylococci. Of 120 milk samples taken after pasteurization (74°C for 15 sec.), 10 contained staphylococci. Finally they reported that 894 staphylococcal strains isolated from workers and cheese samples during the manufacture included 672 coagulase-positive strains, of which 657 produced haemotoxin and 353 lecithinase.

VANSCHOUWENBURG-VANFOEKEN, et al. (1978) found that the 24 -h-old Gouda cheese which contained a number greater than 10^8 enterotoxigenic S.aureus contained enterotoxin, and in one case enterotoxin was already detectable with a number of 3.3×10^7/gm.

DEVRIESE and KERCKHOVE (1979) reported that the strains isolated from pigeons belonging to the coagulase-positive species S.intermedius, coagulase-negative S.hyicus subsp. chromogenes strains from cattle & pig, and S.aureus strains from poultry, gave weakly positive reactions in DNase plate culture and heat-resistant DNase tests but S.aureus and S. intermedius strains from other sources and coagulase-negative and coagulase-positive S.hyicus subsp.hyicus strains reacted strongly in these tests. They proposed also a standardized plate culture test.

LACHICA, et al. (1979) stated that the thermonuclease seroinhibition test is a convenient and reliable means for distinguishing S.aureus from other coagulase-positive staphylococci. A highly specific antiserum against S.aureus TNase is not necessary, commercially available sera produced against staphylococcal toxins or alpha-hemolysin were adequately specific in their inhibition of S.aureus TNase. They also reported that the test may also be helpful in identifying occasional coagulase-negative strains of S. aureus.

PARK, et al. (1979) studied the modification of the method of Tatini, et al. (1976) by addition of non-fat dry milk (NFDM) to food samples and subsequent acid ppt. at pH 3.8 and found that this enhanced the recovery of staphylococcal TNase from most of 37 food tested. They also reported that potato and egg salads, and pastas, all of which gave false-negative results without NFDM.

SHELAIH (1979) examined 105 Egyptian soft cheese samples (70 of Damietta and 35 of Kareish cheeses) collected from different localities in Cairo and Giza and reported that Staph.aureus were isolated from 11.43 and 5.61% of market Dameitta and Kareish cheese samples respectively.

AHMED (1980) examined 700 random samples of raw milk, kareish cheese, Dameitta cheese, Hard cheese, processed cheese, yoghurt, kishk and ice-cream (200, 200, 50, 25, 25,25, 25 and 150 samples of each respectively) collected from Assiut markets, street pedlers and shopkeepers and found that the Staph.aureus was detected in 48.5, 44.5, 26, 4, 36,0,0, and 90% of examined samples respectively. Out of 347 Staph.aureus strains isolated from milk and milk products 228 (65.7%), 228 (65.7%), 165 (47.55%) and 182 (52.45%) strains were positive for coagulase, DNase, egg yolk reaction and haemolysis respectively. He also reported that 91% of enterotoxigenic Staph.aureus strains were positive for coagulase and DNase. Only 3% of enterotoxigenic strains failed to produce coagulase and DNase. Also 3% of enterotoxigenic strains were negative for coagulase and positive for DNase reaction, and 3% of enterotoxigenic strains were vice-versa.

BISSONNETTE, et al. (1980) examined 105 TNase-positive cheese samples comprising 13 types and found that 92 (87.6%) contained coagulase-positive staphylococci.

CARCIA and MORENO (1980) tested 57 staphylococcal strains isolated from clinical and subclinical bovine mastitis for enterotoxigenicity, coagulase and TNase production, mannitol fermentation and lysostaphin sensitivity and found that all strains were coagulase-positive but did not showed the same degree of plasma clotting. 40 cultures were scored as $\overset{+}{4}$, 7 as $\overset{+}{3}$, 7 as $\overset{+}{2}$ and 3 as $\overset{+}{1}$. Also they found that all strains which gave $\overset{+}{4}$ and $\overset{+}{3}$ coagulase reaction showed high sensitivity to lysostaphin and, with one exception, produced TNase. However only 2 of the strains yielding $\overset{+}{2}$ and $\overset{+}{1}$ coagulase reaction possessed these properties. No good correlation was observed between mannitol fermentation and $\overset{+}{4}$ and $\overset{+}{3}$ coagulase reaction, only 4 strains were toxigenic and the enterotoxins produced were C and D.

DANIELSSON, et al. (1980) reported of microbiological investigations on the manufacture of goat's milk cheese on 5 Swedish farms and found that staph. count ranged from $<$ 1000 to 1600000/gm. in freshly made cheeses but was $<$ 100/gm. in virtually all cheeses after 2 months storage. In 3 cheeses (2 made from raw milk and 1 from past. milk) no staphylococci were detected. They also added that staphylococci producing enterotoxin A were found in 4 cheeses.

DEVRIESE and HAJEK (1980) used DNase plate culture test to distinguish and identification of pathogenic staphylococci strains isolated from animals and foods derived

from animals and found that only zones whose widths were at least four times greater than the width of the growth streaks, which should not exceed 2 or 3 mm, were scored as positive. Such DNase test results were typical for strains of S. aureus of human, bovine, porcine and, in some cases, avian origin as were strains of S. hyicus subsp. hyicus and many, but not all S. intermedius strains. They also added that the weak reacting or negative strains of S. aureus occure frequently in poultry.

PARK, et al. (1980) examined a total of 1204 cultures comprising 16 genera for production of TNase in milk and found that 99% of coagulase-positive staphylococci produced TNase and only 18% of the coagulase-negative staphylococci produced the enzyme. Also they reported that the enzyme exhibited a minor peak at pH 7.0 and a broad major peak ranging from pH 8.5 to 10.0. On the other hand they added that there was little loss in activity of the staphylococcal enzyme after 60 min. at 100 °C, and the nuclease produced by coagulase-positive staphylococcus was more heat-stable than the other nuclease.

SINGH, et al. (1980) examined samples of baby foods comprising 7 brands of infant milk and 3 brands of milk-cereal weaning for the incidence of different types of microorganisms, and found that one brand of infant milk food with 91×10^2 organisms also exhibited the maximum number of staphylococci and some of these were coagulase-positive. They also reported that some staphylococcal

isolates showed DNase activity and also produced entero-toxin A or B. On the other hand, one of the reconstituted baby food samples when held at ambient temperature (37.5°C) the S.aureus count increased 10-fold in 3 hours.

ZAADHOF (1980) stated that the most likely source of enterotoxin-producing staphylococci in consumer milk is post-pasteurization contamination by man and/or equipments, and subsequent insufficient cooling. Also he found that the lack of acid development increase the risk of entero-toxin production.

ZAADHOF and TERPLAN (1980) stated that according to their experiences the modification of the method of Tatini, et al. by the addition of non-fat dry milk as recommended by Park, et al. enhances the recovery of TNase and seems to have no longer the disadvantage of false-negative results with regard to some types of food.

FEDER (1981) examined 564 of acid curd cheese samples collected from 12 cheese factories in lower Saxony and found that egg yolk test and/or DNase positive staphylococci were detected in 24 cheese samples.

HEKNEBY and GONDROSEN (1981) examined 2 Mountain cheese samples which caused food-poisoning and found that this were due to Staph.aureus enterotoxin. They also detected characteristic Staph.aureus colonies at 62500/gm. in brined cheese and 28500/gm. in unbrined cheese. Also they could be detected thermostable nucleases which were considered

to be reliable evidence of Staph.aureus food-poisoning.

TESONE, et al. (1981) examined 142 cheese samples and found that the Staph.aureus counts were $\leq 10^2$/gm. in 98.4% of samples.

TODD, et al. (1981) examined 59 Swiss cheese lots and found that most samples had count of $10^4 - 10^6$/gm. of staphylococci. Enterotoxin B was identified in 73% of 186 samples and thermonuclease (TNase) was found in 53% of 122 samples. Also they found variations in Staph. aureus counts enterotoxin and TNase contents and in pH within lots and between the center and periphery of individual cheeses.

PALASUNTHERAM and BEAUCHAMP (1982) tested 293 strains of Staph.aureus isolated from clinical specimens in Srilanka for enterotoxigenicity and found that 39% produced enterotoxins A, B, C, D or E and enterotoxin B was detected more often than the others.

STORPER, et al. (1982) reported that after 2 weeks storage of bovine milk samples at - 18 °C, the viability of isolates of Staph.aureus was reduced by 1.6% and a further reduction in viability ranging between 5 and 20% was recorded following storage for anther 2 weeks.

AHMED, et al. (1983) examined periodically Damietta cheese samples which prepared from unsalted raw milk and raw milk with 5 or 10% Nacl and stored in whey containing

15% Nacl which were held at 30 °C for Staph.aureus count, DNase and salt contents. At the first the milks were inoculated with enterotoxigenic Staph.aureus strain 100 (produces enterotoxin A) befor adding the salt and rennet and found that the numbers of Staph.aureus increased rapidly during preparation of cheese and there was a rapid decrease in the number of viable Staph.aureus during storage of cheese made from unsalted milk and cheese made from milk with 5% added salt. On the other hand, in cheese from milk with 10% added salt, Staph.aureus survived until the fourth week. Also they reported that an increase in salt content and a decrease in the pH value of all cheeses occured during storage in salted whey. Finally they detected DNase only in cheese made from salted milk, but these samples did not contain a detectable amount of staphylococcal enterotoxin either after cheeses were made or stored for one week.

GUDDING (1983) studied the quantity, thermostability and serological pattern of nucleases produced by different staphylococci isolated from 9 different species of animals or from humans and found that S.aureus, S.intermedius, and S.hyicus subsp.hyicus were vigorous producers of nuclease, whereas the coagulase-negative staphylococci, except S. hyicus subsp.hyicus produced significantly less nuclease. He also added that the nucleases of all strains were found to be thermo-stable and S.aureus, S.intermedius and S. hyicus subsp.hyicus could be distinguished from each other

and from coagulase-negative staphylococci on the basis of inhibition of nuclease activity by specific antibodies.

HILL (1983) analyzed and classified 61 thermostable nuclease (TSN) producing staphylococci isolated from raw milk by coagulase tests and found that the most of the S. aureus strains examined were both coagulase and TSN producers. Two isolates (3.3%) which were biochemically S. epidermidis biotype 1 produced bound coagulase and TSN and 7 isolates (11.5%) which were biochemically and by TSN production classified as S.aureus, were unable to produce either free or bound coagulase. From other hand he classified and tested 183 isolates from various dairy sources for the ability to produce TSN and reported that all the 139 isolates identified biochemically as S.aureus produced TSN. Of the 44 isolates which were identified as other species within the family Micrococcaceae, only 2 (4.5%) were TSN producers. Finally he concluded that the TSN test may be used in two ways either in the manner recommended by Rayman et al. (1975) as a method for confirming negative and doubtful coagulase test results, or eventually it may be adopted as a more reliable alternative to the coagulase test itself.

PEREIRA, et al. (1983) studied the effect of temperature, pH and sodium chloride conc. on the production of staphylococcal enterotoxins A and B and found that the highest enterotoxin production was obtained at the optimum growth conditions, 39.4 °C and pH 7.0. They also added that the production of the 2 enterotoxins was completely inhibited at 20 °C, above 45 °C at pH 4.5 and by 12% Nacl.

Increase in the Nacl con.resulted in a decrease in entero-toxins production, with a more pronounced effect on SEB production than on SEA production. Finally they reported that the production of the enterotoxins in 4% Nacl was not observed at pH 6.0 and 6.5 after 18 hours of incubation at 37 °C.

BECKER, et al.(1984) examined 206 samples of commercially available infant food and their ingredients for the incidence of coagulase-positive staphylococci and found that only 2 samples contained coagulase-positive staphylococci (4/gm. of each) .

BECKER, et al. (1984) examined 113, 1, 6, 12 and 14 isolated strains of S.aureus, S.intermedius, S.hyicus subsp. hyicus, coagulase-negative staphylococci and micrococci respectively for production of TNase and found that 100, 100, 100, 33.33 and zero % were produced TNase respectively. Also they reported that the thermonuclease seroinhibition test could be used for distinguishing S.aureus from other coagu-lase-positive staphylococci by using antisera against S. aureus TNase. Finally they concluded that the test can be included in the routine examination of food for S.aureus TNase without considerable expenditure of work and material.

BRODSKY (1984) examined 127 60-day aged raw milk Cheddar and 237 freshly cheese samples produced by 21 and 32 provin-cially inspected cheese plants respectively and found that Staph.aureus was found only in 2 products at a level of

> 1000/gm. and above the screening level of 1000/gm. was not found respectively. He also reported that the results suggested that the producers of Cheddar cheese should have no difficulty in meeting the microbiological standards adopted by the Health Protection Branch, Health and Welfare Canada.

CHUBB, et al. (1985) surveyed bacteriological quality of milk which collected from 3 small goats herds and found that the staphylococcus counts ranged from 5.4×10^{2} to 4.9×10^{4}/ml., and coagulase-positive staphylococci were exist in 86.84%.

MATERIAL AND METHODS

I. Collection and preparation of samples:

150 random samples of milk and milk products (Damietta and Kareish cheeses and Yoghurt) were collected during 1982/83 from different localities in Behera Governorate for the present study work. The samples were directly transferred to the laboratory in Edfina with minimum of delay, where they were prepared for examination.

The samples were tested for the incidence and enumeration of coliforms, enterococci and staphylococci. Respective colonies were picked up and isolated in pure culture for further identification in Edfina laboratory and milk hygiene laboratory in Munich, Faculty of Vet.med. (Federal Republik of Germany). Additionally 20 samples of Bulgarian and Turkish soft sheep cheeses were collected in Munich and tested for the presence of Staphylococcus aureus.

A. Milk samples:

40 random raw milk samples marketed in Behera Governorate were collected from street pedlars, milk shops as well as from Governmental and private farms in clean, dry and sterile stoppered bottles 250 ml. Each sample was thoroughly mixed before being subjected to examination. 10-fold serial dilutions were prepared.

B. Cheese samples:

40 random each of Damietta & Kareish cheeses samples were collected from Behera markets as well as 10 random

each of Bulgarian & Turkish cheese samples from Munich markets. In the laboratory, collected samples were prepared for microbiological examination as follows:

In case of Egyptian samples 11 grams were transferred to a prewarmed sterile morter (40 °C) and triturated with 3 gms. of sterile, dry and fine sand, to which 99 ml. of warm sterile 2% sodium citrate solution at 40 °C were added and thoroughly mixed till completely emulsified to make a dilution of 1 : 10, from which 10-fold serial dilutions were prepared (A.P.H.A., 1972). But in Germany the samples were blended in the mixer at high speed for 1 minute.

C. Yoghurt samples:

30 yoghurt samples, in their containers were collected from milk shops. Each sample was thoroughly homogenized by sterile stirrer. Then 11 gms. of the prepared sample was transferred to a sterile wide-mouth container (beaker), to which 99 ml. of sterile distilled water were added and shaked until a homogeneous dispersion was obtained to make a dilution of 1 : 10, from which 10-fold serial dilutions were prepared. But when homogeneous dispersion could not be prepared, the warm sterile 2% sodium citrate solution at 40 °C was used.

II. Experimental technique:

Each prepared sample was examined for incidence and enumeration of Coliforms, Enterococci and Staphylococci.

A. Coliforms:

1) Enumeration of coliforms: Determination of Most Probable Number:

Into each of three separate fermentation tubes of Lauryl Sulphate Tryptose Broth (ICMSF, 1978) provided with inverted Durham tubes, one ml. of the previously prepared decimal dilutions of the milk, soft cheese and yoghurt samples was inoculated. All inoculated as well as control tubes were incubated at 37°C for 24 hours, after which tubes showing gas were recorded. The tubes without displaying gas were returned to incubator for an additional 24 hrs. After 48 hrs., tubes showing gas production were recorded. The highest dilution in which all three tubes were positive and the next two higher dilutions were selected. If no dilution contained three positive tubes, the three highest dilutions containing positive tubes were selected. If further dilutions beyond that showing three positive tubes were not made, the last three dilutions made were selected. After that it was confirmed that the tubes of Lauryl Sulfate Tryptose Broth previously selected were positive for coliform organisms by transferring a loopful of each to separate tubes of Brilliant-green Lactose Bile 2% Broth. All inoculated tubes as well as control ones were incubated at 37°C for 24 and 48 hrs. The formation of gas confirmed the presence of coliform organisms. The number of tubes in each dilution that were confirmed as positive for coliform organisms were recorded. The most probable number (MPN) of confirmed coliforms/ml. or gm. was determined according to the following Table (ICMSF, 1978).

TABLE 7
Most probable number (MPN) of bacteria; three tubes at each dilution a)

Number of positive tubes at each dilution level							
10^{-1} dilution	10^{-2} dilution	10^{-3} dilution	MPN per g	Confidence limits 99%		95%	
0	1	0	3	<1	23	<1	17
1	0	0	4	<1	28	1	21
1	0	1	7	1	35	2	27
1	1	0	7	1	36	2	28
1	2	0	11	2	44	4	35
2	0	0	9	1	50	2	38
2	0	1	14	3	62	5	48
2	1	0	15	3	65	5	50
2	1	1	20	5	77	8	61
2	2	0	21	5	80	8	63
3	0	0	23	4	177	7	129
3	0	1	40	10	230	10	180
3	1	0	40	10	290	20	210
3	1	1	70	20	370	20	280
3	2	0	90	20	520	30	390
3	2	1	150	30	660	50	510
3	2	2	210	50	820	80	640
3	3	0	200	<100	1900	100	1400
3	3	1	500	100	3200	200	2400
3	3	2	1100	200	6400	300	4800

a) At each dilution level, inoculate 1 ml into each of three tubes of media. To calculate MPN from dilutions greater than those shown, multiply the MPN by the appropriate factor of 10, 100, 1000, etc. For example, if tubes selected come from 10^{-2}, 10^{-3}, and 10^{-4} dilutions, multiply by 10; if from 10^{-3}, 10^{-4}, and 10^{-5} dilutions, multiply by 100.

2- Determination of coliform organisms of faecal origin:

Modified Eijkman test (ICMSF, 1978):

The test was performed on the selected tubes of Laury Sulfate Tryptose Broth that were positive for gas production in the enumeration of coliforms described above as follows (ICMSF, 1978):

10 ml. E.C. Broth tubes provided with Durham tubes were used. The tubes were kept in water-bath adjusted at 44.5°C for at least 24 hrs. before being inoculated with loopful of broth from each of the gas-positive cultures. After that inoculated tubes as well as control ones were returned immediately to the 44.5°C water-bath for 48 hrs. Tubes displaying production of gas in E.C. broth tubes were presumed positive for faecal coliforms.

Afterwards a loopful from each of the positive E.C. Broth tubes was streaked on MacConkey Agar plates so as to obtain discrete colonies after the plates had been incubated for 24 hrs. at 32 ± 1 C. Suspected pink to red colonies were picked up and isolated in pure cultures (semi-solid agar tubes) for the transport to Munich and further identification.

3- Confirmatory tests for coliforms:

Isolated organisms were identify in the milk hygiene laboratory, faculty of Vet.med., Munich university using the following tests.

a) Streaking on Violet-Red-Bile-Lactose-Agar:

At the first Violet-Red-Bile-Lactose-Agar plates were streaked with a loopful from suspected culture from the Semi-

solid Agar culture tubes so as to obtain discrete colonies. After the plates had been incubated for 24 hrs. at 32°C, the suspected colonies were characterised by dark red colour measuring about 0.5mm. or more in diameter with reddish precipitate ring. After that Nutrient Agar plates (oxoid) were streaked by the respective dark red colonies and incubated for 24 hrs. at 32°C for further confirmatory tests.

b) Potassium hydroxide test (GREGERSEN, 1978):

1 or 2 drops of 3% KOH were transferred on a clean and dry glass slide. Then a loopful of the respective culture was mixed in the KOH. After 5-10 sec. stirring the loop was raised and if a thread of slime followed the loop 0.5 - 2 cm. or more the reaction was positive & indicates presence of Gram-negative bacteria, e.g. coliform organisms.

c) Oxidase test (GABY and HADLEY, 1957: GERMAN EGG PRODUCTS REGULATION, 1975):

A 6 cm. square piece of filter paper was put in an empty petridish and impregnated with three drops of a mixture of 1% Alcoholic- Naphthol and 1% Dimethyl-P-phenylenediamine hydrochloride solution (2:3). Then a loopful of the suspected culture was smeared on the filter paper. After that it was left for 2 minutes. If no change in colour reaction occurred, the culture proved to be oxidase-negative.

4. Differentiation of coliforms (IMViC-tests):

For the differentiation of 115 confirmed coliform cultures into species and varieties five tests were carried out referred to collectively as the "IMViC-tests". A grouping of reaction combinations is presented in Table 2.

TABLE 2
Differentiation of coliforms

	Gas in lactose bile-salt medium at 44-45.5°C	Indole test	Methyl red test	Voges-Proskauer test	Growth in citrate
Escherichia coli					
Type I (typical)	+	+	+	−	−
Type II	−	−	+	−	−
Intermediates					
Type I	−	−	+	−[b]	+
Type II	−	+	+	−[b]	+
Enterobacter[c] *aerogenes*					
Type I	−	−	−	+	+
Type II	−	+	−	+	+
Enterobacter cloacae	−	−	−	+	+
Irregular					
Type I	−	+	+	−	−
Type II	+	−	+	−	−
Type VI	+	−	−	+	+
Irregular, other types	Reactions variable				

a This table is similar to the one given in Ministry of Health ... (1957).
b Weak positive reactions are occasionally found.
c The Judicial Commission of the International Committee on Nomenclature of Bacteria of IAMS has officially substituted the term *Enterobacter* as the generic designation for the genus previously known as *Aerobacter* (International Association of Microbiological Societies, 1963).

a) Production of gas at 44.5 °C:

10 ml. of Brilliant-green Lactose Bile 2% Broth tubes provided with Durham tubes were used. The tubes were inoculated with a loopful from the pure suspected culture. After that the inoculated tubes as well as control ones were immediately incubated at 44.5 °C for 48 hrs. in the adjusted water-bath. The tubes were examined after 24 hrs. and 48 hrs. for gas production. Formation of gas within the Durham tube was presumed as positive.

b) Indole test (ISO/DIS 7251, 1983):

10 ml. of Tryptone Water tubes were used. The tubes were inoculated from the pure suspected organism. After that the inoculated tubes as well as control ones were incubated immediately in the 44.5 °C water-bath for 24 hrs. then 0.5ml. of Kovacs reagent was added to the tube which after shaking was allowed to stand for 10 minutes. A dark red colour in the amyl alcohol surface layer constitutes a positive indole test.

c) Methyl red test (GERMAN EGG PRODUCTS REGULATION, 1975):

Each pure suspected isolate was inoculated in two tubes each containing 5 ml. of Methyl red-Voges Proskauer Broth (MR-VP Broth). After that one of them as well as control ones were incubated at 37°C for 4 days. The other one as well as control ones were incubated at room temperature (21°C) for 4 days. Then to each tube 5 drops of alcoholic methyl red solution were added. The formation of red colour

indicates a positive result.

d) Voges-Proskauer test (GERMAN EGG PRODUCTS REGULATION, 1975):

Each respective organism was inoculated in two tubes each containing 5 ml. of MR-VP Broth. One of them as well as control ones were incubated at 37°C for 4 days, While the other tube as well as control ones were incubated at room temperature (21°C) for 4 days. After that 3 ml. of BARRITT reagent and 1 ml. of Potassium hydroxide 40% were added to each tube. Colour change indicates a positive result.

e) Citrate utilization test (SIMMONS, 1926):

Simmons Citrate Agar plates were streaked with respective organisms and incubated as well as control ones at 37°C for 48 hrs. Growth, which is indicated by a change in colour of the medium to blue, is recorded as positive.

5- Identification of irregular coliform types:

28 cultures were irregular types in the "IMViC-pattern" (see Table 2). These cultures were additionally tested with Enterotube II (Roche), a system allowing the simultaneous examination of 15 different biochemical reactions. For the interpretation of the results the so-called computer code- and identification-system for Enterotube II was used.

B. Enterococci:

1. Enumeration of enterococci:

Many selective plating media were developed and evaluated for enumeration and isolation of enterococci, but the Enterococcus Selective Differential medium (ESD) of EFTHYMIOU et al., (1974) proved to be of value not only for enumeration but also for relatively rapid presumptive differentiation of enterococci and yielded a fast rate of growth and maximum colony size. After preparation of medium, it was poured into plates, allowed to solidify and stored in poly-ethylen bags in refrigerator. For enumeration of enterococci 0.1ml. from each previously prepared dilution was spread evenly on the surface of the medium by a sterile bent glass rod. Inoculated plates were incubated at 37 °C for 48 hrs. Magenta, pink and white colonies representing E.faecalis, intermediate strains and E.faecium respectively, were counted according to EFTHYMIOU et al.,(1974).

2. Isolation of enterococci:

SF-Broth (HAJNA and PERRY, 1943) tubes were inoculated with suspected colonies of enterococci from ESD as a selective medium for the growth only of Group D streptococci. After incubation at 37°C for 24 to 48 hrs., the presence of an acid reaction and a colour change from purple to yellow was presumptive evidence for the presence of the Group D streptococci species. Subculture were made on the Citrate Azide Agar (A.P.H.A., 1972), which were incubated at 37°C for no more than 72 hrs. Representative colonies (blue colour)

were inoculated in Semi-solid Agar tubes for transport to Munich and further identification.

3. Confirmatory tests for enterococci:

a) Streaking on Kanamycin-Esculin-Azide Agar(MOSSEL et al.,1978):

At the first a loopful of respective organism was streaked on Kanamycin-Esculin-Azide Agar (Oxoid) plates, which were incubated at 37°C for up to 72 hrs. The plates were examined after and the formation of black haloes around the colonies indicated a positive result for Lancefield Group D streptococci. After that a loopful of suspected organism from Kanamycin-Esculin-Azide Agar plates was streaked on 0.02% Esculin-Cow Blood-Agar and incubated for 24 - 48 hrs. at 37°C for detection of haemolysis and production of pure culture. Subsequently Brain-Heart-Infusion Broth tubes each containing 5 ml. were inoculated with a loopful from Blood Agar plates and incubated at 37°C for 18 - 24 hrs. for further confirmatory tests.

b) Morphological characteristics:

Films were prepared from Brain-Heart-Infusion Broth on clean and dry glass slides, then covered by glass cover and examined microscopically for the presence of cocci, mostly in pairs or chains.

c) Biochemical characteristics:

ca-Catalase test:

About 1 ml. of each of the BHI Broth cultures was obtained and mixed in another tube with 1 ml. of 3% Hydrogen

peroxide (tube method). Additionally a loopful of respective culture was transfered on a clean and dry glass slide as well as a loopful of 3% Hydrogen peroxide, which were mixed together. Enterococci are catalase-negative, e.g. air bubbles do not appear.

cb-Modified benzidine test (DEIBEL and EVANS, 1960):

The suspected cultures which were not clear in the catalase reaction, were streaked on Plate Count Agar. After that all plates were incubated at 37°C for 24 - 48 hrs. Then at the first the plates were flooded with the Benzidine dihydrochloride solution followed by the addition of an approximately equal volume of 5% Hydrogen peroxide. A positive reaction indicated by appearance of blue-green to deep blue coloration quickly, could not be observed. Enterococci are benzidine-negative.

d)Physiological characteristics:

The criteria of SHERMAN (1937) were tested in this part of examination.

da-Growth at 45 C and 10 C:

Tempered Brain-Heart-Infusion Broth (BHI) tubes each containing 5 ml. were inoculated with the isolated suspected organisms. After that the inoculated tubes were incubated at 44 °C up to 48 hrs. in adjusted water-bath or up to 14 days at 10 °C (refrigerator). The presence of growth indicated a positive result for enterococci.

db- Growth at 6.5% sodium chloride (SHERMAN, 1937):

BHI Broth with 6.5% NaCl tubes each containing 5 ml. were inoculated with respective organisms and incubated at 37 °C for 72 hrs. The positive result for enterococci is indicated by growth.

dc- Growth at pH 9.6 (SHERMAN, 1937)

Isolated and respective organisms were inoculated into BHI Broth tubes adjusted at pH 9.6, after that the inoculated tubes were incubated at 37 °C for 3 days. The tubes were examined daily and the presence of growtn indicated a positive result for the enterococci.

e) Serological characteristics:

The precipitation technique (LANCEFIELD, 1933) was adopted for the streptococci grouping, by using available group D antisera of Wellcome.

4. Identification of Enterococcus faecalis:

Potassium tellurite (0.04%), 2,3,5-Triphenyltetrazoliumchloride (0.01%) and sugars as well as API 20 Strep were used for the identification of Enterococcus faecalis (see also Table 3).

a) Potassium tellurite tolerance:

The isolated confirmed organisms were streaked on the Plate Count Agar containing 0.04% potassium tellurite (SKADHAUGE, 1950). After that all inoculated plates were incubated at 37°C for 3 days. E.faecalis can grow i.e

tolerate the potassium tellurite (black colonies).

b) 2,3,5-Triphenyltetrazoliumchloride (0.01%) reduction:

The isolated confirmed organisms were streaked on Heart-Infusion Agar plates containing 0.01% of 2,3,5-TTC and 1% D (+) dextrose (FACKLAM, 1973). The streaked plates were incubated at 37 °C for 3 days. The positive result is indicated by deep magenta coloured colonies (E.faecalis)and the negative result indicated by colourless or faintly pink colonies (E.faecium, E.durans).

c) Sugar fermentation reaction:

Isolated organisms were inoculated into Purple Broth Base (RAJ et al., 1961; TILTON and LITSKY, 1967; STARK,1970) containing 1% of the required sugar L (+) Arabinose, D (-) Sorbitol and D Tagatose. The Purple Broth tubes were shaken gently and all tubes as well as one control were incubated at 37°C and the reaction of inoculated tubes were noticed every 24 hrs., for 5 successive days. The positive reaction was indicated by appearance of yellow colour. A prolonged incubation up to 30 days may be required to confirm a negative result.

d) API 20 STREP (api-bio Merieux):

Some of the isolated organisms which were not clear by the biochemical differentiation tests were tested by what's called API 20 STREP test. The inoculation, incubation and identification were done according to api-bio Merieux, the latter using the analytical profile index.

Table 3: Differentiation of Enterococcus (E.) faecalis, E. faecium and E. durans (SCHLEIFER und KILPPER-BÄLZ, 1984; COLLINS et al., 1984)

Characteristic	E. faecalis	E. faecium	E. durans
Reduction of:			
0.04% tellurite	+	–	–
0.01% tetrazolium	+	–	–
Acid produced from:			
L-Arabinose	–	+	–
D-Sorbitol	+ (–)	–	–
D-Tagatose	+	–	–

5. Production of thermonuclease (TNase) (LACHICA et al., 1971 and PARK et al., 1980):

The production of TNase by microorganisms other than S. aureus has been reported for enterococci (THOMAS and NAMBUDRIPAD, 1974 and MEDWID, 1978).

a) Thermonuclease test:

The cultures were grown on cow blood agar and incubated at 37 °C for 24 hrs. After that a loopful of culture was transferred to 100 ml. of ultra high-heated skim milk (0.3% fat) in 250 ml. flask as well as to BHI Broth tubes. All flasks and tubes were incubated at 37 °C for 3 days. Then in case of the skim milk the pH was adjusted to 3.8 with 1 N HCL and the suspension was centrifuged at 18000 rpm for 20 min. at 4 °C. After that the supernatant was treated with 0.05 volumes (4 - 5ml) of cold 3 m Trichloroacetic acid (TCA) and left for 15 min. in the refrigerator, then recentrifuged at 18000 rpm for 20 min. at 4 °C. Then the ppt. was resuspended in a final volume of 2 ml 0.05 m sterile Trisbuffer (pH 9.0) and adjusted to pH 8.5 with 2 N NaoH. After that this solution as well as BHI Broth tubes were heated in a boiling water-bath for 15 min. and left to cool. Finally 7 ul with fine pipette from each boiled sample were placed in the precut wells (2 mm), tempered at 37 °C for 0.5 - 1 hour of Toluidine blue-O-DNA-Agar plates (pH 6.7 and 9.0). All plates were incubated at 37 °C and the results were obtained after 4, 24 and 48 hrs.respectively (BECKER et al., 1984). The heat-stable nuclease activity is indicated

by bright pink zones of DNA hydrolysis.

b) Thermonuclease seroinhibition test (BECKER et al., 1984)

It was found that the thermonuclease seroinhibition test was a simple, convenient and reliable means for distinguishing S.aureus TNase from other TNases. So in this work it was used for detection of TNase group D streptococci producers.

For this purpose pairs of wells spaced 4 mm apart were cut into the Toluidine blue-O-DNA Agar. By fine pipette (7 ul), one well of each pair was filled with S.aureus thermonuclease-antiserum, and the other well was filled with boiled enzyme extract (from skim milk). After that each plate was incubated at 37 °C for 1 to 4 hrs., and the inhibition was indicated by the flattening of the bright pink halo of thermonuclease activity at the area near the well containing the serum. From other hand serial twofold dilutions of the antisera were made to determine the highest dilution at which inhibition was still demonstrable.

C. Staphylococci:

1. Enumeration of S.aureus:

0.1 ml. amount from each of prepared dilutions of samples under investigation was transferred and evenly spread over a dry surface of the selective agar media using surface streaking technique by a sterile bent glass rod (CLARK and NELSON, 1961; THATCHER and CLARK, 1975). Baird-Parker Agar and modified Baird-Parker Agar plates (HOLBROOK et al., 1969) were streaked and incubated at 37 °C for 24 hrs. Suspected colonies (black, shiny with narrow white margin and surrounded by clear zones extending into the opaque medium) were counted. Then the plates were reincubated for another 24 hrs. before being counted again for further growth. The two media were matched for the count of presumptive S. aureus, but there was no significant difference between counts of freshly poured plates of the original Baird-Parker medium and modified medium, as the colony size and area of clear zones around the colonies were also similar. So the use of the original medium was adopted in this work.

2. Isolation of S.aureus:

10% Sodium chloride broth tubes (BAILEY and SCOTT, 1974) were inoculated with suspected colonies of S.aureus from Baird-Parker medium. All inoculated tubes were incubated at 37 °C for 48 hrs. The presence of growth is considered positive for staphylococci. After that a loopful from inoculated 10% NaCl broth tubes was streaked on Mannitol Salt

Agar. The inoculated plates were incubated at 37 °C for 24 hrs. Suspected colonies (surrounded by a yellow halo) were picked up and for further studies inoculated in Semi-solid Agar tubes which were incubated at 37 °C for 24 hours.

3. Confirmatory tests for staphylococci:

a) Streaking on Baird-Parker Medium:

A loopful of suspected organism was inoculated into tempered BHI Broth tubes each containing 5 ml. After that the inoculated tubes were incubated at 37 °C for 24 hrs. and a loopful from BHI tubes was streaked on Baird-Parker Agar plates and incubated at 37 °C for 24 hrs., then the culture was examined for typical colonies.

b) Streaking on Blood Agar:

A loopful from BHI tubes was streaked on 0.02% Esculin Cow Blood Agar plate which was incubated at 37 °C for 48 hrs., then the culture was examined for growth and haemolysis.

c) Microscopical examination:

Films were prepared from the Blood Agar on a clean and dry glass slide, then covered by glass cover and examined microscopically for the presence of cocci occurring in clusters.

d) Catalase test:

A loopful of fresh 3 % H_2O_2 was transfered on a clean and dry glass slide as well as a loopful of respective culture, which were then mixed together. The formation of gas

bubbles is typical for staphylococci.

4. Identification of Staphylococcus aureus:

a) Coagulase-test:

To a wasserman tube containing 0.3 ml. of Bacto coagulase plasma with EDTA (rabbit plasma., Difco, 0803 - 47) 0.1 ml. from overnight BHI Broth culture were added, another tube with plasma was left as a control. After that both tubes were incubated in water-bath at 37 °C for 4 hrs., and examined periodically for coagulation by gently tipping the tube after 15 min.during the first hour and once every hour thereafter until 4 hours had elapsed. Finally all tubes not positive were reincubated and examined after 24 hours.

b) Slide agglutination technique:

On the clean and dry glass slide one drop of Bacto coagulase plasma with EDTA (rabbit plasma., Difco, 0803 - 47) as well as loopful from the suspected organism were mixed carefully together. The positive result for S.aureus is indicated by the quickly formation of clear clumping (detection of bound coagulase).

c) Staphyslide test (api-bio Merieux):

The staphyslide test was used to confirm S.aureus as follows:

On a rigorously clean and dry glass slide one drop of sensitized red cells (R_1) as well as one drop of control red cells (R_2) were transferred and to each of the drops one or two of suspected colonies which were grown on the Blood Agar

were added and the mixture was mixed carefully with a platinum loop. The presence and appearance of clear clumping (agglutination) within 15 seconds in the sensitized red cell suspension (R_1) only indicates the presence of S.aureus.

5. Detection of deoxyribonuclease:

a) Thermonuclease test (LACHICA et al., 1971):

Overnight BHI Broth cultures were heated, in a boiling water-bath, for 15 min. from other hand Toluidine blue-O-DNA-Agar was prepared and about 12.5 ml.quantities were pipetted into petridishes. After solidification, wells, 2 mm in diameter were cut in the agar, and the agar plugs were removed with a metal canula. Aliquots of the preheated suspected cultures as well as control ones (7 ul) were added in the precut wells using fine pipette. After that all plates were incubated at 37 °C (sometimes at 50 °C in a moist chamber) for 4 hrs. and the heat stable nuclease activity is indicated by bright pink zones of DNA hydrolysis around the wells.

b) Thermonuclease seroinhibition test (BECKER et al., 1984):

It was found that the thermonuclease seroinhibition test was a simple, convenient and reliable means for distinguishing S.aureus from other coagulase-positive staphylococci.

The molten Toluidine blue-O-DNA Agar (TDA) was poured into petridishes to a depth of 2 mm; when gelled, pairs of wells spaced 4 mm apart were cut into the agar. By fine

pipette (7 µl), one well of each pair was filled with thermonuclease-antiserum against S.aureus, and the other well was filled with boiled BHI culture. After that each plate was incubated at 37 °C for 1 to 4 hrs., and the inhibition was indicated by the flattening of the bright pink halo of TNase activity at the area near the well containing the serum. From other hand serial twofold dilutions of the antisera were made to determine the highest dilution at which inhibition was still demonstrable.

c) Deoxyribonuclease plate culture test (DEVRIESE and VAn DE KERCKHOVE, 1979):

The production of heat-stable or heat-labile deoxyribonuclease (DNases) is not a unique property of S.aureus. So a standardized procedure should be used to distinguish the staphylococci which produce strong DNase effects from weakly reacting staphylococci.

The strains were cultured in Nutrient Broth No. 2 (Oxoid) and incubated at 37 °C for overnight. Then with inoculation needles of different diameters the strains were streaked on the plates with DNase Agar (Oxoid) of a constant volume of the medium (20 ml in 90 mm petridishes). After incubation at 37 °C minimally for 20 hrs. and not longer than 28 hrs., The cultures were flooded with N HCl and the diameters of growth spots or streaks, and clear reaction zones whose widths are at least four times greater than the width of the growth streak, which should not exceed 2 or 3 mm, are scored as positive. Such DNase test results are typical for S.aureus of human, bovine, porcine and, in some cases, avian origin

as well as S.hyicus subsp.hyicus and S.intermedius strains from dogs.

6. Detection of enterotoxigenicity:

As some S.aureus strains produce enterotoxin, it is very important to identify the enterotoxigenic staphylococci.

a) Enterotoxin production (DONNELLY et al., 1967):

100ml of double strength BHI Broth was placed inside a sac made from a piece of dialysis tubing approximately 4.5cm. wide by 65 to 70 cm long. The tube was placed in a 1000 ml. Erlenmeyer flask in a U-shape and the two knotted ends, located in the neck of the flask, were secured with plaster band. After that the flask was autoclaved at 121 °C for 15 min., and 10 ml of sterile phosphate-buffered saline were added to the flask, outside the sac of medium. From other hand the cultures were streaked on Blood Agar and incubated at 37°C for 18 - 24 hrs. before it was used to inoculate sac-culture assemblies. The growth on the Blood Agar was harvested with 6 and 4 ml of sterile phosphate-buffered saline two times respectively, and was added outside the sac of medium to provide an inoculum volume of 20 ml. After that the flask was incubated at 37°C on a rotary shaker at 100 rpm for 48 hrs. Finally the growth surrounding the sac was removed from the flask, and centrifuged at 10.000 rpm (4 °C) for 30 min. and the supernatant fluid was analyzed for enterotoxin.

b) Serological assay of enterotoxin (SOMMERFELD and TERPLAN, 1975):

The precipitation tests are of high sensitivity and

technical simplicity and offer quantitative or qualitative results after a short period. So the detection of the enterotoxins (A, B, C_1, C_2 and D) in the culture supernatant fluids was accomplished with the modified microslide gel double diffusion test.

Three glass slides were placed on the Immuno frame (each frame holds six glass slides and is placed on the Immunoleveling table set and holded by the Immuno frame holder and secured by locking clampes) and were flooded with 10 ml of liquid gel solution (1.2% Agrose and 1% of polyethylene glycol 6000 gel buffer). The plate slides were left for 15 min. for gelling, then in moist container in the refrigerator for at one hour before using. After that the punch set was placed on the gel-covered glass slides one after one, and subsequently wells of about 1.5 - 2 mm diameter were obtained and the excess of the gel was removed by the suction needle 1.2 mm diameter. The each two opposite wells were filled by using fine pipette with 7 ul of the reference antigen as well as the supernatant fluid from the S.aureus culture being examined respectively. From other hand the center hole was filled with 7 ul of suitable and desired diluted antiserum. After that the slides were incubated in a moist chamber at 37 °C for 18 to 24 hrs. Then the plate slides were covered with 3 moistened filter paper strips one over another and dried at 50 - 60 °C for 2 - 3 hrs. Then the plate slides were stained by Coomassie-Brilliant-blue-solution for 5 min. and rinsed in the warm (50 °C) stain removal solution for 1 min. two times. Finally the plate slides were dried

and examined. The tests in which the lines of ppt. from the unknown coalesced with the reference line of ppt. (formed by the reaction of the antiserum with the reference antigen) were considered positive for the enterotoxin.

D. Acidity of yoghurt samples (LING, 1956):

10 grams of well mixed sample was weighed in a clean and dry porcelain dish and diluted with 20 ml. of dist. CO_2 free water. One ml. of 1% phenolphthalein solution was added. After that the contents were titrated against N/9 NaoH solution to the first persistant faint pink shade. Finally the acidity % was calculated according to the following formula: Acidity % = $\frac{R}{10}$. R = No of ml. of N/9 NaoH used in titration.

E. Sodium chloride content of cheese samples (Chemi. & test. of Dairy products, 1977):

The method used is that after the American Dairy Science Association Committee on chemical methods for the analysis of milk and dairy products as follows:

Three grams of the cheese samples (Damietta and Kareish) were weighed and ground in a conical flask and wetted with 30 ml. of 0.1711 N silver nitrate solution. After that 15ml. of halogen-free pure nitric acid as well as 50 ml. of dist. water were added. The flask was boiled. As the mixture boiled, approximately 15 ml. of saturated potassium permanganate solution in 5 ml. portions were added, then boiled until all cheese particles were digested. After that the solution was diluted to about 100 ml. The liquid was decanted into a

beaker, and the precipitate was washed by adding 100 ml. of distilled water and decanted again. 3 ml. of a saturated ferric ammonium sulfate was added as an indicator. The excess of silver nitrate was titrated against 0.1711 N potassium sulfocyanate. The sodium chloride content was calculated according to the following formula:

NaCl % = number of ml. of silver nitrate used (30)-titration value (R)/ weight (3 grams).

F. Staphylococcus aureus and thermonuclease activity in sheep cheese:

In Munich 10 random samples each of Bulgarian and Turkish sheep cheese have been collected on the market and tested for the presence of Staphylococcus aureus and thermonuclease.

For the enumeration of Staphylococcus aureus the samples were prepared in the described manner (section I B), spread over the dry surface of Baird-Parker-Agar plates and incubated at 37°C for 24 and 48 hrs (section II C 1). After enumeration of typical colonies these and suspect atypical ones (black colonies without egg yolk reaction) were subjected to the coagulase test.

Moreover the sheep cheese samples were tested for thermonuclease (PARK et al., 1979). To 20 gms cheese 5 gm. of non-fat-dry milk which was free from TNase and 2 volumes (50 ml) of deionized water were added. The sample was blended in the mixer at high speed for 1 minute and the pH was adjusted to 3.8 with 1 N HCl. After that the suspension was centrifuged at 18000 rpm for 20 min. at 4 °C and the

supernatant fluid was obtained and treated with 0.05 volumes (2 ml) of cold 3 m Trichloroacetic acid (TCA). This mixture was left for 10 - 15 min. then recentrifuged for another time at 18000 rpm for 20 min. at 4 C. After that the precipitate was resuspended in a final volume of 2.0 ml. in 0.05 m sterile Trisbuffer (pH 9.0) and adjusted to pH 8.5 with 4 N NaoH. Finally this solution was heated in a boiling water-bath for 15 min. and about 10 ul with fine pipette was placed in the precut wells (2 mm) of Toluidine blue-O-DNA-Agar plates. All plates were incubated at 37°C for 4 and 24 h. in a moist chamber and the heat stable nuclease activity was indicated by bright pink zones of DNA hydrolysis.

Table 4.: Statistical parameters of coliform count (MPN/ml or gm) in milk, Damietta cheese, Kareish cheese and yoghurt samples

Parameters	Type of food (number of samples examined)			
	Milk (40)	Damietta cheese (40)	Kareish cheese (40)	Yoghurt (30)
Minimum	<3	<3	<3	4×10^3
Maximum	$>1.1 \times 10^8$	$>1.1 \times 10^8$	$>1.1 \times 10^8$	$>1.1 \times 10^8$
$\bar{x}_a$	4.05×10^6	4.71×10^6	1.57×10^7	4.38×10^6
S.E.M.	2.79×10^6	2.81×10^6	4.77×10^6	3.66×10^6
C.V.	0.04	0.04	0.04	0.02
$\bar{x}_g$	5.61×10^3	1.85×10^3	7.53×10^5	1.15×10^5

Table 5:
Frequency distribution of coliform counts (MPN/ml or gm) in milk, Damietta cheese, Kareish cheese and yoghurt

Coliform count (per ml or gm)	Milk		Damietta cheese		Kareish cheese		Yoghurt		All products	
	No. of samples	%	No. of samples	%	No. of samples	%	No. of samples	%	No. of samples	%
< 3	3	7.5	8	20.0	1	2.5	0	0	12	8.0
3-9	3	7.5	8	20.0	0	0	0	0	11	7.3
$\geq 10^1$	5	12.5	2	5.0	2	5.0	0	0	9	6.0
$\geq 10^2$	8	20.0	3	7.5	2	5.0	0	0	13	8.7
$\geq 10^3$	7	17.5	3	7.5	3	7.5	9	30.0	22	14.7
$\geq 10^4$	5	12.5	5	12.5	4	10.0	12	40.0	26	17.3
$\geq 10^5$	3	7.5	4	10.0	7	17.5	5	16.7	19	12.7
$\geq 10^6$	5	12.5	6	15.0	14	35.0	3	10.0	28	18.7
$\geq 10^7$	1	2.5	1	2.5	7	17.5	1	3.3	10	6.6
Total	40	100	40	100	40	100	30	100	150	100

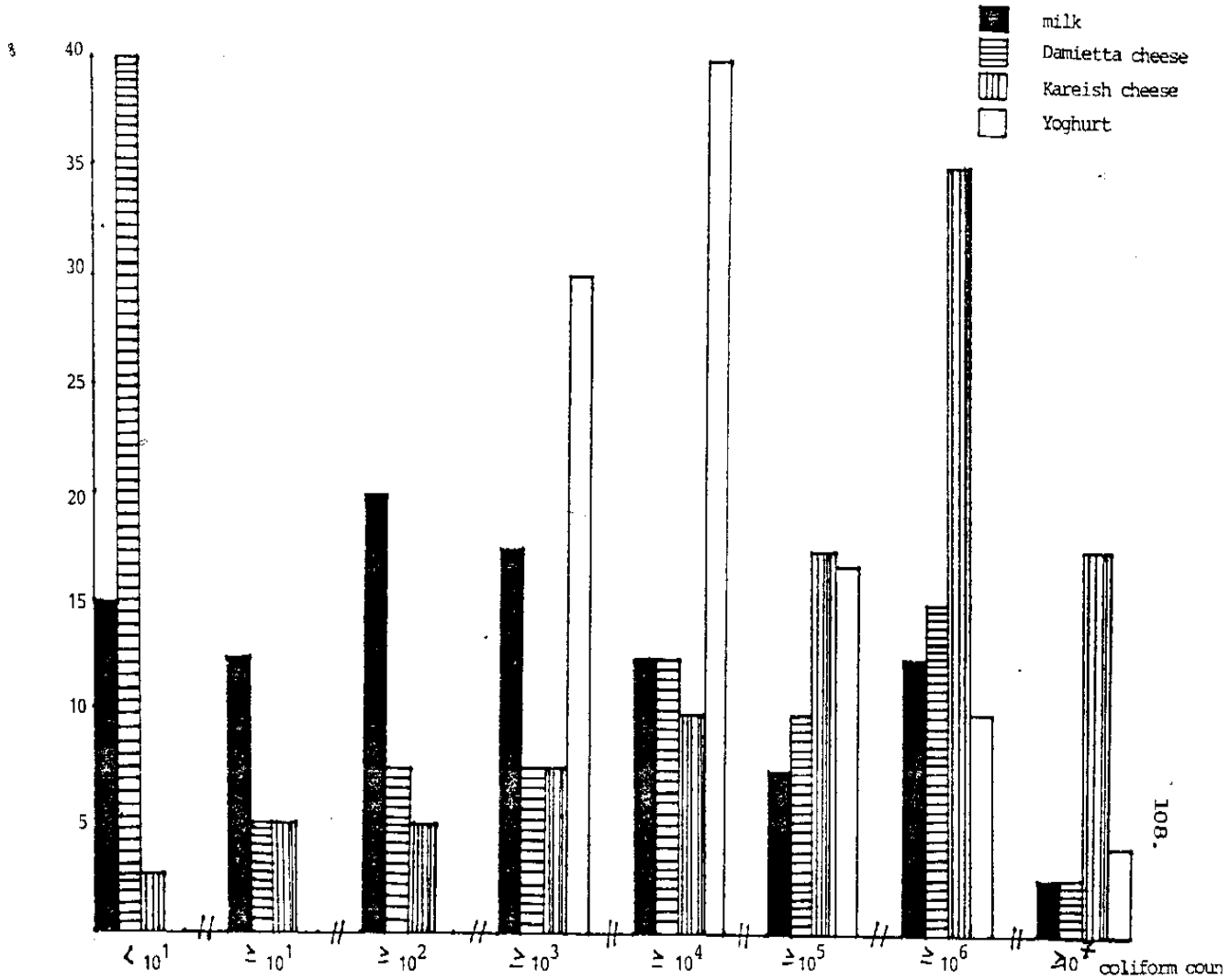

Figure 1: Histogram of examined milk, Damiette cheese, Kareish cheese and youghurt samples based on their coliform count (MPN/ml orgm.)

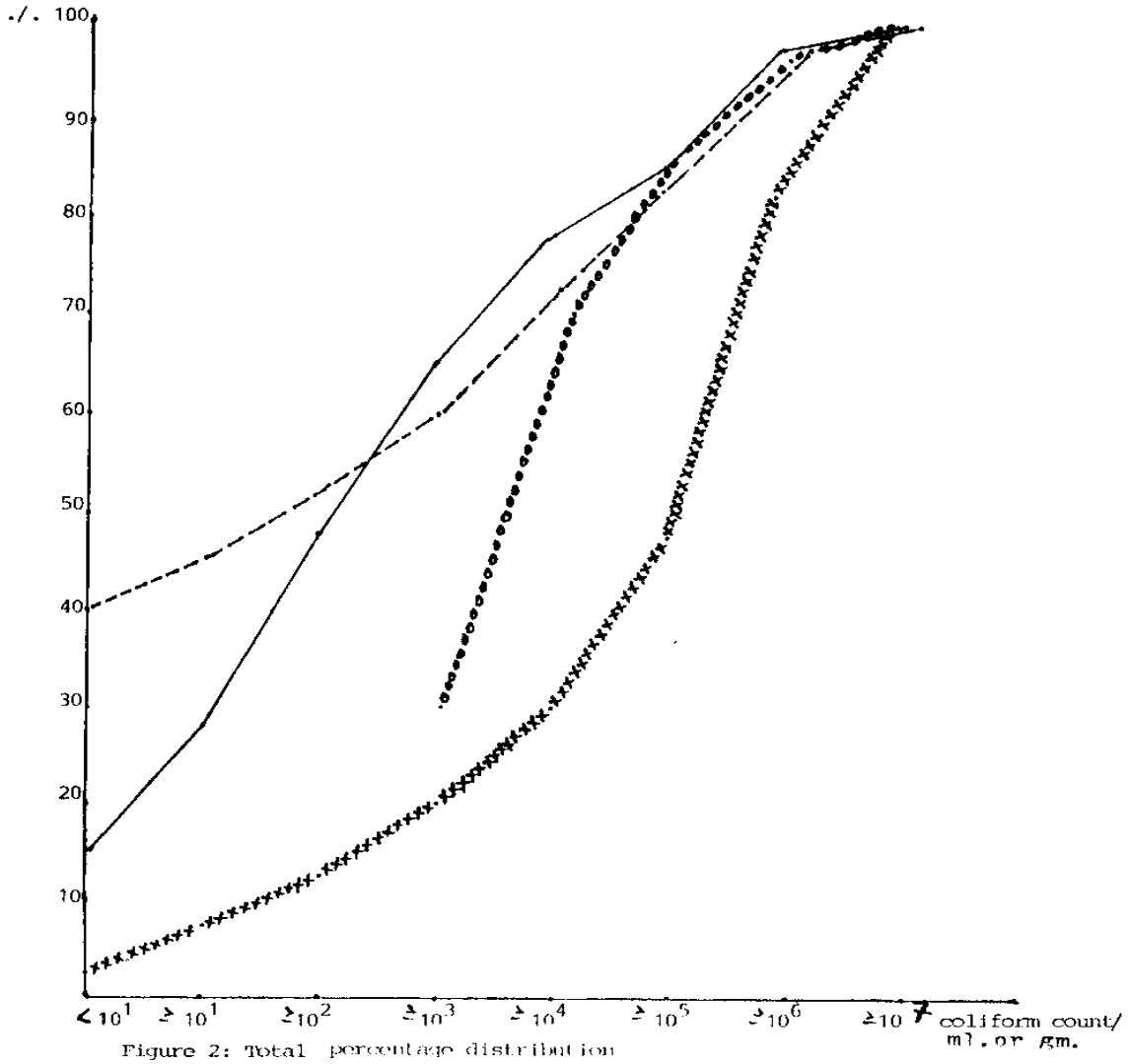

Figure 2: Total percentage distribution

Table 6: ANOVA Table for simple linear regression of examined Damietta cheese samples based on their coliform count/gm.

S.o.v.	D.F.	S.S.	M.S.	$F_{cal.}$	$F_{tab.}$
Total s.s.	39	123751178.5	-		
					4.08
S.S. due to regression	1	11577184.9	11577184.9	3.92	
					7.31
S.S. from regression	38	112173993.6	2951947.2		

S.o.v. = Source of variation
D.F. = degree of freedom
S.S. = Sum square
M.s. = Mean square
F.cal. = calculated F.
F.tab. = tabulated F.

Level of significance at 0.05
0.01

Table 7: ANOVA Table for simple linear regression of examined Kareish cheese samples based on their coliform count/gm

S.o.v.	D.F.	S.S.	M.S.	$F_{cal.}$	$F_{tab.}$
Total s.s.	39	3550834.15			
					4.08
S.S. due to regression	1	136958.97	136958.97	1.52	
					7.31
S.S. from regression	38	3413875.18	89838.82		

Level of significance at 0.05
0.01

Table 8: ANOVA Table for simple linear regression of examined youghurt samples based on their coliform count/gm.

S.o.v.	D.F.	S.S.	M.S.	$F_{cal.}$	$F_{tab.}$
Total s.s.	29	116677053			
					4.17
S.S. due to regression	1	2255896.41	2255896.41	0.55	
					7.56
S.S.from regression	28	114421156	4086469.86		

Level of significance at 0.05
0.01

Table ...9.: Differentiation of 115 "thermotrophic" coliform strains isolated from milk and milk products using the IMViC-tests (ICMSF, 1978)

Type of strain	number (percentage) of strains				
	Milk	Damietta cheese	Kareish cheese	Yoghurt	Total
E. coli Type I	23 (74.2)	12 (66.7)	31 (79.5)	21 (77.8)	87 (75.7)
Irregular Type I	0	5 (27.8)	8 (20.5)	2 (7.4)	15 (13.0)
Irregular Type II	3 (9.7)	0	0	4 (14.8)	7 (6.1)
Irregular, other types	5 (16.1)	1 (5.5)	0	0	6 (5.2)
Total	31 (100)	18 (100)	39 (100)	27 (100)	115 (100)

Table 10: Differentiation of 28 coliform strains of irregular type according to IMViC tests using Enterotube II

Type of strain (IMViC pattern)	No. of strains	Species (No. of strains)
Irregular Type I (-++--)	15	Escherichia coli (15)
Irregular Type II (+-+--)	7	Escherichia coli (7)
Irregular, other types (Reactions variable)	6	Escherichia coli (2) Klebsiella pneumoniae (3) Enterobacter cloacae (1)

Table .11: Statistical parameters of enterococcus count (ml or gm) in milk, Damietta cheese, Kareish cheese and yoghurt samples

Parameter	Type of food (number of samples examined)			
	Milk (40)	Damietta cheese (40)	Kareish cheese (40)	Yoghurt (30)
Minimum	< 10	1×10^3	7×10^4	4×10^2
Maximum	3.3×10^7	8.1×10^7	8×10^7	2×10^6
$\bar{x}_a$	1.59×10^6	9.56×10^6	8.61×10^6	2.14×10^5
S.E.M.	8.83×10^5	2.9×10^6	2.7×10^6	8.75×10^4
C.V.	0.04	0.02	0.02	0.02
$\bar{x}_g$	8.44×10^3	1.18×10^5	2.29×10^6	3.60×10^4

Table 12:

Frequency distribution of enterococcus counts (ml or gm) in milk, Damietta cheese, Kareish cheese and yoghurt

Enterococcus count (per ml or gm)	Milk		Damietta cheese		Kareish cheese		Yoghurt		All products	
	No. of samples	%	No. of samples	%	No. of samples	%	No. of samples	%	No. of samples	%
$< 10^1$	7	17.5	0	0	0	0	0	0	7	4.7
$\geq 10^1$	0	0	0	0	0	0	0	0	0	0
$\geq 10^2$	6	15.0	4	10.0	0	0	8	26.7	18	12.0
$\geq 10^3$	5	12.5	14	35.0	1	2.5	11	36.6	31	20.7
$\geq 10^4$	13	32.5	9	22.5	6	15.0	8	26.7	36	24.0
$\geq 10^5$	6	15.0	3	7.5	21	52.5	3	10.0	33	22.0
$\geq 10^6$	2	5.0	6	15.0	11	27.5	0	0	19	12.6
10^7	1	2.5	4	10.0	1	2.5	0	0	6	4.0
Total	40	100	40	100	40	100	30	100	150	100

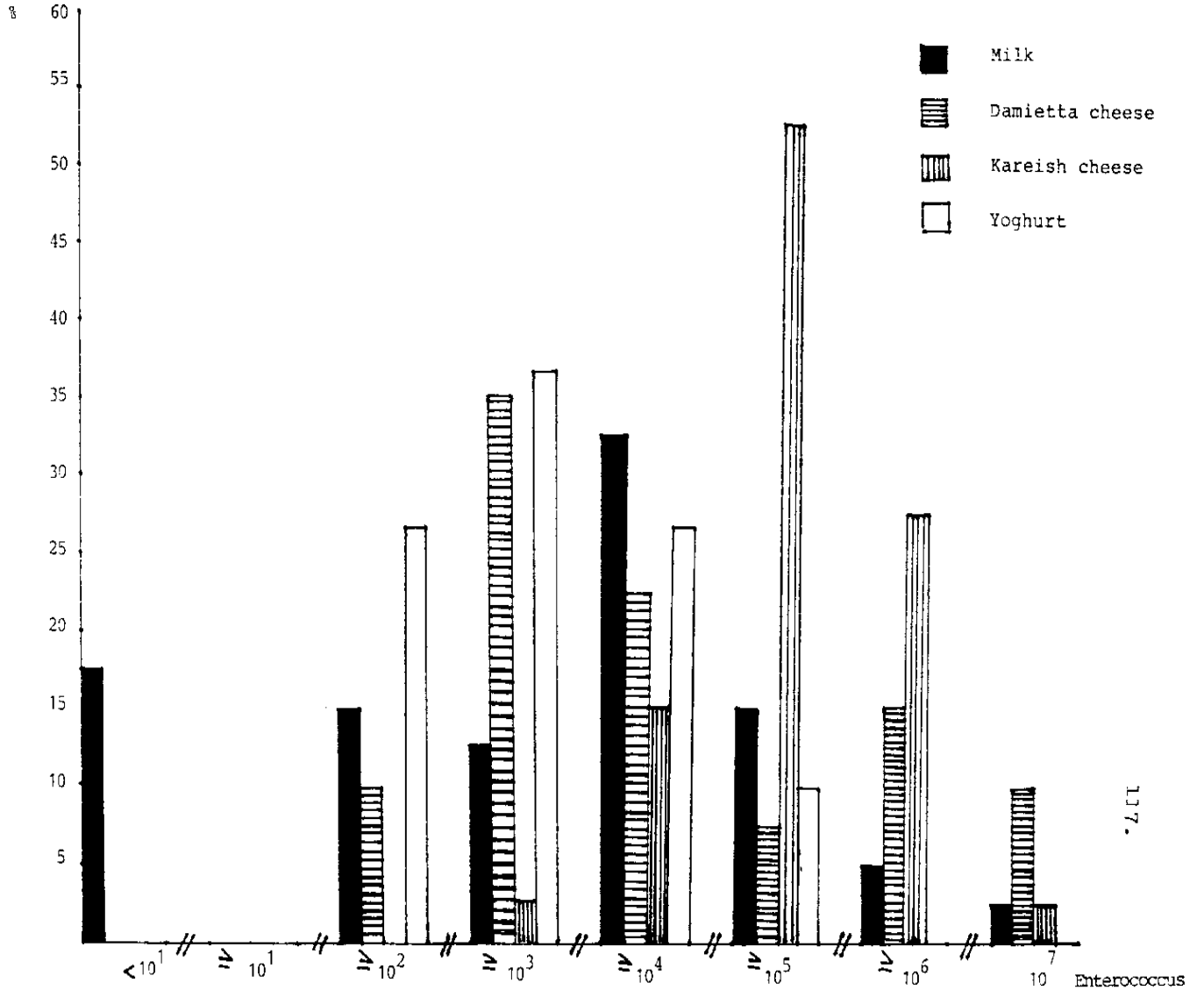

Figure 3: Histogram of examined milk, Damietta cheese, Kraeish cheese and Yoghurt samples based on their enterococcus count/ ml or gm.

Figure 4: Total percentage distribution.

Table 13: ANOVA Table for simple linear regression of examined Damietta cheese samples based on their enterococcus count/ml. or gm.

S.o.V.	D.F.	S.S.	M.S.	$F_{cal.}$	$F_{tab.}$
Total s.s.	39	131330209			
					4.08
S.S. due to regression	1	38629900.3	38629900.3	15.84	
					7.31
S.S. from regression	38	92700308.7	2439481.8		

Level of Significance at 0.05

0.01

Table 14: ANOVA Table for simple linear regression of examined Kareish cheese samples based on their enterococcus count/ml or gm.

S.o.V.	D.F.	S.S.	M.S.	$F_{cal.}$	$F_{tab.}$
Total s.s.	39	1139866.25			
					4.08
S.S. due to regression	1	9119.12	9119.12	0.31	
					7.31
S.S. from regression	38	1130747.13	29756.5		

Level of significance at 0.05

0.01

Table 15: ANOVA Table for simple linear regression of examined Yoghurt samples based on their enterococcus count/ml. or gm.

S.o.V.	D.F.	S.S.	M.S.	$F_{cal.}$	$F_{tab.}$
Total s.s.	29	66544.92			
					4.17
S.S. due to regression	1	2804.01	2804.01	1.23	
					7.56
S.S. from regression	28	63740.91	2276.46		

Level of Significance at 0.05

0.01

Table 16: Differentiation of 129 enterococci strains isolated from milk, Damietta cheese, Kareish cheese and Yoghurt

Type of strain	number (percentage) of strains				
	Milk	Damietta cheese	Kareish cheese	Yoghurt	Total
Enterococcus faecalis	21 (67.7)	24 (64.9)	23 (67.7)	22 (81.5)	90 (69.8)
Enterococcus faecium	7 (22.6)	12 (32.4)	6 (17.6)	5 (18.5)	30 (23.2)
Enterococcus durans	3 (9.7)	1 (2.7)	5 (14.7)	0 (0)	9 (7.0)
Total	31 (100)	37 (100)	34 (100)	27 (100)	129 (100)

Table 17: Statistical parameters of presumptive Staphylococcus aureus count (ml or gm) in milk, Damietta cheese, Kareish cheese and yoghurt samples

Parameter	Type of food (number of samples examined)			
	Milk (40)	Damietta cheese (40)	Kareish cheese (40)	Yoghurt (30)
Minimum	1.2×10^3	8×10^2	8×10^2	3×10^3
Maximum	1.7×10^7	1×10^7	7.5×10^8	2.2×10^6
$\bar{x}_a$	1.39×10^6	1.8×10^6	6.87×10^6	3.04×10^5
S.E.M.	5.43×10^5	4.56×10^5	2.84×10^6	1.02×10^5
C.V.	0.03	0.02	0.03	0.02
$\bar{x}_g$	1.29×10^5	1.09×10^5	1.44×10^5	3.69×10^4

Table 18:

Frequency distribution of presumptive Staphylococcus aureus counts (ml or gm) in milk, Damietta cheese, Kareish cheese and yoghurt

Staphylococcus count (per ml or gm)	Milk		Damietta cheese		Kareish cheese		Yoghurt		All products	
	No. of samples	%	No. of samples	%	No. of samples	%	No. of samples	%	No. of samples	%
$< 10^1$	0	0	0	0	0	0	0	0	0	0
10^1	0	0	0	0	0	0	0	0	0	0
$\geq 10^2$	5	12.5	5	12.5	9	22.5	4	13.3	23	15.3
$\geq 10^3$	10	25.0	10	25.0	5	12.5	15	50.0	40	26.7
$\geq 10^4$	14	35.0	7	17.5	8	20.0	4	13.3	33	22.0
$\geq 10^5$	8	20.0	7	17.5	8	20.0	7	23.4	30	20.0
$\geq 10^6$	3	7.5	8	20.0	8	20.0	0	0	19	12.7
$\geq 10^7$	0	0	3	7.5	2	5.0	0	0	5	3.3
Total	40	100	40	100	40	100	30	100	150	100

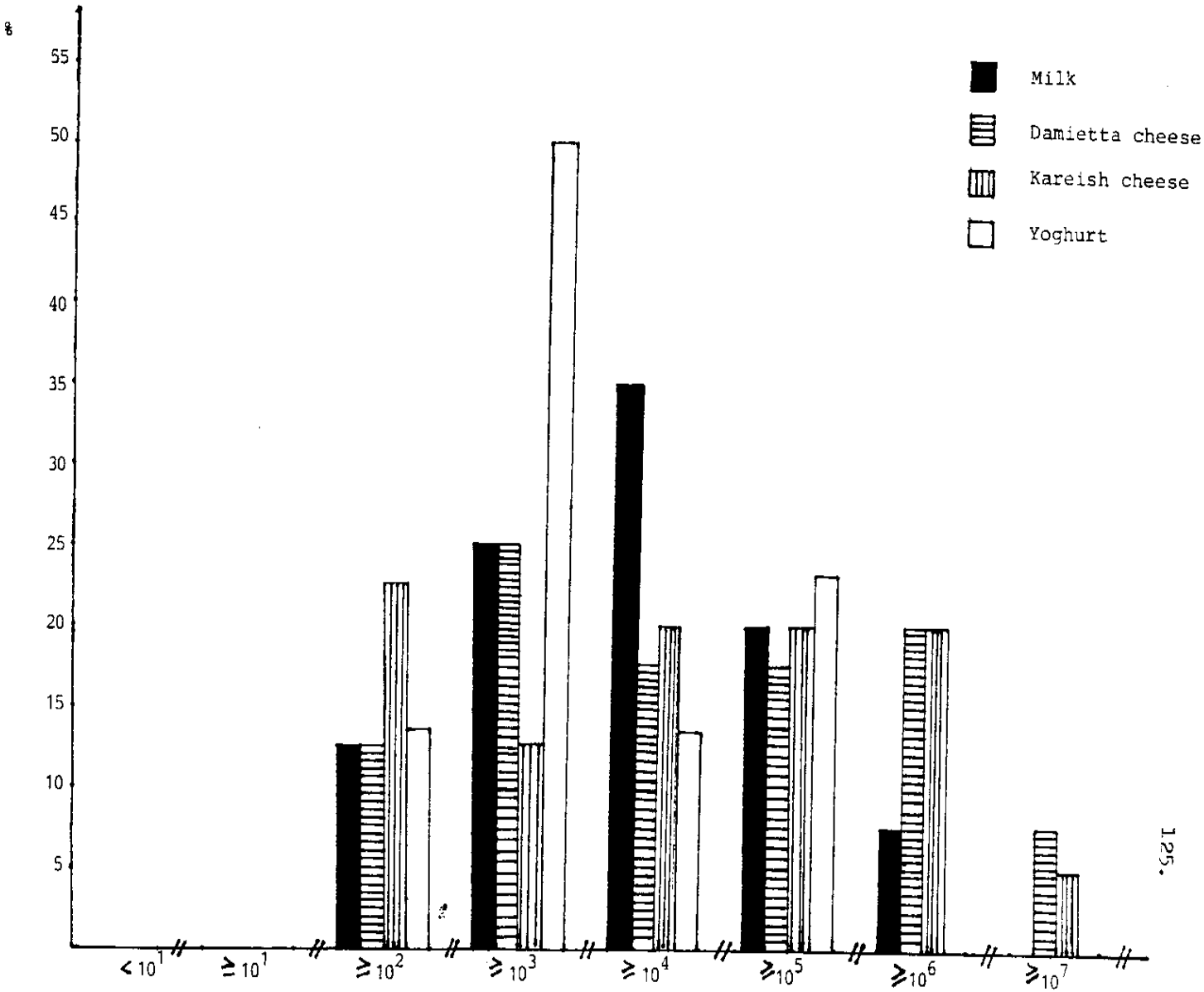

Figure 5: Histogram of examined milk, Damietta cheese, Kareish cheese and Yoghurt samples based on their presumptive S. aureus count/ml or gm.

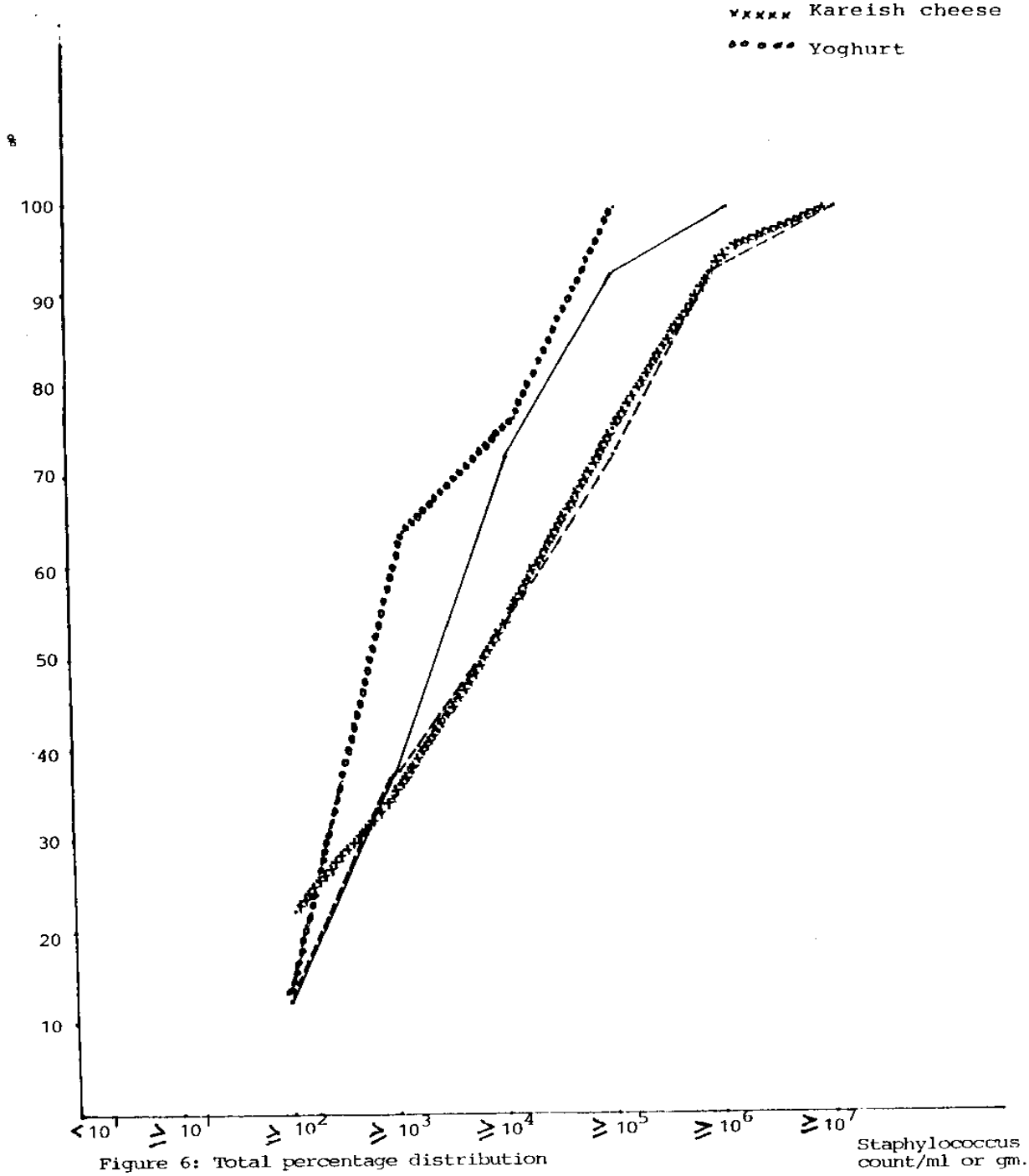

Figure 6: Total percentage distribution

Table 19: ANOVA Table for simple linear regression of examined Damietta cheese samples based on their presumptive Staphylococcus aureus count/ml or gm

S.o.V	D.F.	S.S.	M.S.	$F_{cal.}$	$F_{tab.}$
Total s.s.	39	32475.15			
					4.08
S.S. due to regression	1	8139.15	8139.15	12.71	
					7.31
S.S. from regression	38	24336.00	640.42		

Level of Significance at 0.05

0.01

Table 20: ANOVA Table for simple linear regression of examined Kareish cheese samples based on their Staphylococcus count/ml or gm

S.o.V.	D.F.	S.S.	M.S.	$F_{cal.}$	$F_{tab.}$
Total s.s.	39	125882385			
					4.08
S.S. due to regression	1	22209.77	22209.77	0.00671	
					7.31
S.S. from regression	38	125860175	3312109.87		

Level Significance at 0.05

0.01

Table 21: ANOVA Table for simple linear regression of examined Yoghurt samples based on their Staphylococcus count/ml or gm

S.o.V.	D.F.	S.S.	M.S.	$F_{cal.}$	$F_{tab.}$
Total s.s.	29	89813.45			
					4.17
S.S. due to regression	1	12294.72	12294.72	4.44	
					7.56
S.S. from regression	28	77518.75	2768.53		

Level of Significance at 0.05

0.01

Table 22: Identification of presumptive Staphylococcus aureus strains isolated from milk, Damietta and Kareish cheese and Yoghurt samples

Type of food	Number of examined samples	Number of isolated strains	Number of S. aureus strains	%
Milk	40	35	16	45.7
Damietta cheese	40	30	7	23.3
Kareish cheese	40	37	5	13.5
Yoghurt	30	23	8	34.8
Total	150	125	36	28.8

Table 23: Reaction of Staphylococcus aureus and enterococci in the thermonuclease-seroinhibition-test (according to BECKER et al., 1984)

Tested organism	Number of strains	Number of TNase - positive strains	Number of strains reacting with antiserum
Staphylococcus aureus	36	36	36
Enterococci	129	10	0

Table 24: Reaction of Staphylococcus aureus strains in the Deoxyribonuclease plate culture test (according to DEVRIESE and VAN DE KERCKHOVE, 1979)

Type of food	Number of strains examined	Ratio of diameters of growth and reaction 1: <4	%	1: 4-6	%	1:>6	%
Milk	16	0	0	15	93,8	1	6,3
Damietta cheese	7	0	0	6	85,7	1	14,3
Kareish cheese	5	0	0	5	100	0	0
Yoghurt	8	0	0	8	100	0	0
Total	36	0	0	34	94,4	2	5,6

Table 25: Statistical parameters of sodium chloride content (Damietta cheese and Kareish cheese) and acidity (yoghurt)

Type of food	Number of samples examined	Minimum	Maximum	Range	Mean	S.E.M.	C.V.
Damietta cheese	40	2.93	9.23	6.30	6.52	0.37	0.36
Kareish cheese	40	0	9.3	9.3	2.95	0.39	0.83
Yoghurt	30	0.48	14.5	14.02	3.71	0.84	1.24

DISCUSSION

Incidence of coliforms:

The coliforms and E.coli are used as indicators of the bacteriological quality of raw milk and milk products as well as the efficiency of hygiene in farm milking parlours, dairy factories and shops. On the other hand a little attention is given to the possible hazards to public health of these microorganisms.

Results given in Table 4 show that the Most Probable Number of coliforms/ml in the examined 40 milk samples reached from < 3 to 1.1×10^{8} with an arithmetic mean ($\bar{x}_a$) of 4.05×10^{6} and a geometric mean ($\bar{x}_g$) of 5.61×10^{3}. 47.5% of the samples contained less than 10^{3} coliforms/ml (Table 5; Figures 1 and 2), 77.5% less than 10^{5} coliforms/ml. In 15% of the samples at least 10^{6} coliforms were found.

Nearly similar findings were reported by EL-SADEK & HAMED (1957), ITO (1963), BECK (1965), KIELWEIN (1966), YANKOV (1967), ANON (1967), ANON (1970), GHAZVINIAN, et al. (1972), KALINA, et al. (1973), OZALP (1974), SHELAIH (1976), EL-ESAWY (1978), MOUSTAFA (1978) and MOHAMED (1981). Comparatively lower results were recorded by FIRAUX, et al. (1952), IMAMURA, et al. (1958), KYLA & ANNA (1962), BOTTAZZI (1966), AHMED, et al. (1974) and GAHLOT, et al. (1975). The lowest counts were reported by ROMAIN & ESSAFI (1968), KUMAWAT, et al. (1972) and CHUBB, et al. (1985).

The 40 Damietta cheese samples showed an arithmetic mean of 4.71×10^{6}/gm. The minimum number was lower than

3/gm, the maximum number higher than 1.1×10^8/gm (Table 4). 40% of the samples had MPN of less than 10^1/gm and 17.5% of more than 10^5/gm (Table 5; Figures 1 and 2). The Kareish cheeses showed higher contents of coliforms, though minimum (<3/gm) and maximum ($>1.1 \times 10^8$/gm) didn't differ. The artithmetic mean was 1.57×10^7 coliforms/gm, the geometric one 7.53×10^5 /gm. Only 20% of Kareish cheeses contained less than 10^4 coliforms/gm and still 52.5% at least 10^6 coliforms/gm (Table 4 and 5; Figures 1 and 2).

Results obtained were in agreement to a certain extent with those reported by GHONEIM (1963), MOURSY & NASR (1964), DEVOYOD, et al. (1968), MARTINEZ & FERNANDEZ (1978), SHELAIH (1979), BURZYNSKA (1980), LUCK & DUNKELD (1981) and WOOD, et al. (1983). However BRAG & KAMPE (1969), PARK, et al. (1973), FAHMY & YOUSSEF (1974), COLLINS-THOMPSON, et al. (1977), FRANK & MARTH (1977), OTTOGALLI, et al. (1979), FEDER (1981) and TESONE, et al. (1981) reported to some extent lower results, while LYONS & MALLMANN (1954), MANOLKIDIS, et al. (1974), IKONOMOV, et al. (1976) and BRODSKY (1984) recorded lowest results.

There was no significant relation between the coliform MPN and the sodium chloride content of both types of cheese (Tables 6 and 7).

In the yoghurt samples the Most Probable Number ranged from 4×10^3/gm to $>1.1 \times 10^8$/gm. The arithmetic mean run to 4.38×10^6 coliforms/gm, the geometric mean to 1.15×10^5 coliforms/gm. The majority of the samples (70%) contained

10^3 or 10^4 coliforms/gm, but 13.3% had still more than 10^5 coliforms/gm (Tables 4 and 5; Figures 1 and 2).

Comparatively lower results were reported by HUDEC (1968), but the lowest results were obtained by VARABIOFF (1979). On the other hand CERAN (1971), TZANETAKIS (1972), PAPAVASSILIOU, et al. (1973) and KIELWEIN (1980) reported only that coliforms were found in examined yoghurt samples.

From the ANOVA-Table (Table 8) it can be seen, that there was no significant relation between acidity % and coliform MPN.

The faecal E.coli type I proved to exist in 74.2,77.8, 79.5 and 66.7% of the examined milk, yoghurt, Kareish cheese and Damietta cheese respectively (Table 9), while irregular type I proved to exist in 0.0, 7.4, 20.5 and 27.8% respectively. The irregular type II was found in 9.7, 14.8, 0.0 and 0.0%, the irregular other types were found in 16.1,0.0, 0.0 and 5.5% of previously examined milk and milk product samples respectively.

The gained results in examined milk samples were nearly similar with those obtained by MOUSTAFA (1978), while comparatively lower values were recorded by TOVAR (1955), YANKOV (1967) and ROMAIN & ESSAFI (1968). The lowest results were reported by MURRAY (1960), HALL, et al. (1967), JONES (1971), AHMED, et al. (1974), MOUSTAFA, et al. (1975), EL-ESAWY (1978), SINCH & RANGANATHAN (1978) and JOHNSTON, et al. (1982). However BROOKS (1974) recorded higher values.

In examined yoghurt samples comparatively similar findings were reported by PAPAVASSILIOU, et al. (1973) while HALL, et al. (1967) and TZANETAKIS (1972) recorded lower results. Higher value was reported by VARABIOFF (1979).

The obtained results from examined Kareish cheese were in agreement to a certain extent with those reported by ABD-EL-RAHMAN (1972) and LUCK & DUNKELD (1981). Comparatively lower values were recorded by HALL, et al. (1967), BROOKS (1974), MANOLKIDIS, et al. (1974) and EL-BASSIONY (1977). However lowest results were obtained by REINNEKE (1965), FANTASIA, et al. (1974), SINGH & RANGANATHAN (1974), TZANETAKIS, et al. (1977), SHELAIH (1979) and BRODSKY (1984). On the other hand MOURSY & NASR (1964) reported higher values.

In examined Damietta cheese samples nearly similar findings were reported by BROOKS (1974) and MANOLKIDIS, et al. (1974). Lower results were obtained by GHONEIM (1963), REINNEKE (1965), HALL, et al. (1967), ABD-EL-RAHMAN (1972), FAHMY & YOUSSEF (1974), FANTASIA, et al. (1974), SINGH & RANGANATHAN (1974), TZANETAKIS, et al. (1977), SHELAIH (1979) and BRODSKY (1984), but higher value was reported by LUCK & DUNKELD (1981).

The differentiation of the 28 strains which were classified as irregular types according to the IMViC pattern showed that all strains belonged to the types I and II were E.coli, whereas only two of the six strains of other irregular types were also E.coli (Table 10).

Although various aspects of coliform bacteria in milk

and milk products have been studied, comparatively little information is available on the occurrence of pathogenic serotypes of E.coli in dairy products. It appears that wherever faecal coliforms occur, there is also a chance that enteropathogenic E.coli (EEC) will be found.

E.coli is a normal inhabitant in the gut of man and animals. Therefore their presence in milk and milk products may be indicative of faecal contamination. Moreover, these organisms can grow readily in milk and milk products, especially in summer, resulting in undesirable changes in milk and its products. From a public health point of view, entero-pathogenic serotypes have been implicated in cases of fatal gastroenteritis in children and infants as well as in summer diarrhea. Recently E.coli has attracted much attention as a potential pathogen suspected to be associated with outbreaks of gastro-enteritis and food poisoning in human beings. (GORBACH et al., 1971; MATSIEVSKII et al., 1971; FANTASIA et al., 1974).

Incidence of enterococci:

The presence of intestinal inhabitants should be taken as indicative of lack of cleanliness. So the group D streptococci could serve effectively as sanitary indicators of faecal contamination and food quality.

The results given in Table 11 show that the arithmetic mean was 1.59×10^6/milk and the geometric mean was 8.44×10^3/ml milk. The counts reached from < 10/ml (enterococci nondetectable) to 3.3×10^7/ml. 75% of the milk samples were in the range from $10^2 - 10^5$/ml (Table 12; Figures 3 and 4).

The gained results were nearly similar to a certain extent with those obtained by OZALP (1974), GOGOV (1975), EL-ESAWY (1978), OTTOGALLI, et al. (1979), MOHAMED (1981) and BRAG (1982). On the other hand JICINSKA & PESEK (1967), HASHIMOTO (1968), KUMAWAT, et al. (1972), THOMAS & LAXMINARAYANA (1972), KALINA, et al. (1973), MARIMUTHU, et al. (1975), AHMED & EL-BASSIONY (1978) and MOUSTAFA (1978) reported lower counts.

In Damietta cheese samples the counts varied from 1×10^3/gm to 8.1×10^7/gm. The arithmetic mean was 9.56×10^6 whereas the geometric mean was 1.18×10^5/gm (Table 11). 67.5% of the samples had enterococcus counts up to 10^4/gm, but 25% contained at least 10^6/gm (Table 12; Figures 3 and 4). For Kareish cheese the following results have been recorded: $\bar{x}_a$: 8.61×10^6/gm, $\bar{x}_g$: 2.29×10^6/gm; range from 7×10^4/gm to 8×10^7/gm (Table 11). In 82.5% of the Kareish cheese samples counts of at least 10^5/gm were registered (Table 12; Figures 3 and 4).

Values obtained were in agreement to a certain extent with those obtained by HANNAY & NEWLAND (1951), CLARK & REINBOLD (1966), ALEKSIEVA (1979), OTTOGALLI, et al. (1979), ALEKSIEVA (1980), DAVE, et al. (1980), RAMOS, et al. (1981), BRAG (1982) and MUCCHETTI, et al. (1982), while BRAG & KAMPE (1969), AHMED (1977), AHMED & EL-BASSIONY (1978) and SHELAIH (1979) reported lower results.

The yoghurt samples had a minimum count of 4×10^{2}/gm and a maximum of 2×10^{6}/gm (Table 11). The arithmetic mean was 2.15×10^{5}, the geometric mean was 3.60×10^{4}/gm. The highest frequency distribution (90%) was in the range from 10^{2}/gm to 10^{4}/gm (Table 12; Figures 3 and 4).

Nearly similar findings were recorded by BRAG (1982), while AHMED & EL-BASSIONY (1978) reported lower results.

Table (13) reveals that the F.cal. was more than F.tab. (F.ratio at 0.05 & 0.01) so the quantitative relationship between the salt % and enterococci content in examined Damietta cheese samples is significant. On the contrary Table (14) shows that the F.cal. was less than F.tab. (F. ratio at 0.05 & 0.01) so that the quantitative relationship between the salt % and enterococci content in examined Kareish cheese samples is not significant and may be attributed to accidentality. In Table (15) the F.cal. was less than F.ratio at 0.05 & 0.01) so the quantitative relationship between the acidity % and enterococci content in examined yoghurt samples is not significant and may also be attributed to accidentality.

In Table (16) the Ent.faecalis, Ent.faecium and Ent. durans proved to exist in 67.7, 22.6 and 9.7% of the examined milk samples, in 64.9, 32.4, 2.7% and 67.7, 17.6 and 14.7% of the examined Damietta and Kareish cheeses samples respectively as well as in 81.5, 18.5 and 0% of the examined yoghurt samples.

In milk samples nearly similar findings for Ent.faecalis were reported by FACKLAM (1971), KALINA, et al. (1973), AHMED & EL-BASSIONY (1978) and MALIK (1982). However HASHIMOTO, et al. (1964), JANOSSY (1969), AHMED, et al. (1974), MOUSTAFA, et al. (1975), BRUM, et al. (1977), PAPAVASSILEIOU & STAMATELOPOULOU (1977) and CARRASCO, et al. (1978) recorded lower values. A comparatively higher value was obtained by SOLBERG, et al. (1957)., while for Ent.faecium the recorded result was in agreement to a certain extent with that reported by MALIK (1982)., however STOCKER (1958), HASHIMOTO, et al. (1964) and CARRASCO, et al. (1978) reported higher results. On the other hand lower values were obtained by FACKLAM (1971). Nearly similar results for Ent.durans were reported by MALIK (1982), while HASHIMOTO, et al. (1964) and FACKLAM (1971) reported lower values, but CARRASCO, et al. (1978) reported to a certain extent higher results.

The gained results obtained from examined Damietta cheese for Ent.faecalis were similar those obtained by SOLBERG, et al. (1957), FACKLAM (1971), MALIK (1982) and SUAREZ, et al. (1983). However GHONEIM (1963), CLARK & REINBOLD (1966), JANOSSY(1969), AHMED (1977), EL-BASSIONY (1977), DAMDINSUREN & GRUEV (1978)

and DAVE, et al. (1980) reported lower values. Higher results were obtained by DUCHENNE, et al. (1969), AHMED & EL-BASSIONY (1978), SHELAIH (1979) and MUCCHETTI, et al. (1982). For Ent. faecium a comparatively equal result was recorded by ALEKSIEVA (1979), but BALDOVIN-AGAPI, et al. (1963), ALEKSIEVA (1980) and DAVE, et al. (1980) reported higher values. Lower results were obtained by DUCHENNE, et al. (1969), FACKLAM (1971), MALIK (1982), MUCCHETTI, et al. (1982) and SUAREZ, et al.(1983). On the other hand the recorded values for Ent.durans were lower than those obtained by BALDOVIN-AGAPI, et al. (1963), FACKLAM (1971), DAVE, et al. (1980) and MALIK (1982), while DAMDINSUREN & GRUEV (1978), ALEKSIEVA (1979), ALEKSIEVA (1979), ALEKSIEVA (1980) and SUAREZ, et al. (1983) recorded very higher results.

The obtained values from examined Kareish cheese samples for Ent.faecalis were in agreement to a certain extent with those recorded by SOLBERG, et al. (1957), FACKLAM (1971) and MALIK (1982), on the other hand DUCHENNE, et al. (1969), AHMED & EL-BASSIONY (1978), SHELAIH (1979) and MUCCHETTI, et al. (1982) reported higher results, while GHONEIM (1963), CLARK & REINBOLD (1966), JANOSSY (1969), AHMED (1977), EL-BASSIONY (1977), DAMDINSUREN & GRUEV (1978), DAVE, et al. (1980) and SUAREZ, et al. (1983) reported lower values. The recorded results for Ent.faecium were comparatively similar with those obtained by DUCHENNE, et al. (1969)., but BALDOVIN-AGAPI, et al. (1963), ALEKSIEVA (1979), ALEKSIEVA (1980), DAVE, et al. (1980), MALIK (1982) and MUCCHETTI, et al. (1982) recorded higher results. On the other hand

FACKLAM (1971) and SUAREZ, et al. (1983) reported lower values. For Ent.durans recorded BALDOVIN-AGAPI, et al. (1963), FACKLAM (1971), DAVE, et al. (1980) and MALIK (1982) lower results, while DAMDINSUREN & GRUEV (1978), ALEKSIEVA (1979 & 1980) and SUAREZ, et al. (1983) reported higher values.

In examined yoghurt samples FACKLAM (1971) recorded lower results for Ent.Faecalis and Ent.faecium but a higher result for Ent.durans. AHMED & EL-BASSIONY (1978) reported a lower value for Ent.faecalis, while MALIK (1982) recorded higher results for Ent.faecium and Ent.durans but lower value for Ent.faecalis.

The thermonuclease production from isolated strains of group D streptococci was detected in 32.26, 0.0, 0.0 and 0.0% of examined milk, Damietta cheese, Kareish cheese and yoghurt samples respectively, while the TNase-seroinhibition against S.aureus nuclease antiserum for the positive TNase strains was negative (Table 25). All TNase-positive strains were Ent.faecalis.

The gained results were nearly similar with those recorded by BATISH, et al. (1982)., while BISSONNETTE, et al. (1980), PARK, et al. (1980) reported lower values. On the other hand BECKER, et al. (1984) reported no positive result.

Although these streptococci are not of great clinical importance, they are one of the most studied groups of streptococci. This is because of their distribution and importance in water, food and dairy industries, where their

presence can be correlated with faecal contamination from human and animal sources. In addition to their use as pollution indicators, it would be advantageous to employ these organisms to trace the sanitary history of a food products so that preventive measures could be initiated in order to minimize or prevent contamination. The enterococci are widely distributed in foods, they decompose lactose to lactic acid which induce certain undesirable changes in milk and its products, grow in a wide range of temperature (between 7 - 10 and 45°C) and pH (between 4 and 8 - 10), and are tolerant to salts (6.5% Nacl), therefore the enterococci are of interest due to their characteristics of being the most thermo-resistant among the non-sporulated microorganisms and provide a good general index of faecal contamination as well as food quality and Ent.faecalis is believed to be a specific index of human pollution.

Incidence of Staphylococcus aureus:

The presence of viable staphylococci (S. aureus) in food initially and their subsequent growth during processing and/or storage could lead to presence of various enterotoxins capable of causing food poisoning. On the other hand the staphylococci are important not only in staphylococcal food poisoning but also in many human infections. Therefore staphylococci continue to be an important problem for food processors, food service works, and consumers.

The results given in Table 17 show, that the arithmetic mean for presumptive S. aureus (black colonies with halo on Baird-Parker Agar) was 1.39×10^6/ml milk, whereas the geometric one was 1.29×10^5/ml. The counts reached from 1.2×10^3/ml to 1.7×10^7/ml. Nearly three quarters of the milk samples (72.5%) had presumptive S. aureus counts within the range 10^2 - 10^4/ml (Table 18; Figure 5 and 6).

Results obtained were in agreement to a certain extent with those recorded by CLARK & NELSON (1961), SHARPE, et al. (1965) and ABO-EL-NAGA (1972), while LENGAUER & STUMTNER (1974), SINGH, et al. (1980), BECKER, et al. (1984) and CHUBB, et al. (1985) reported lower values.

In Damietta cheese samples the counts varied from 8×10^2/gm to 1×10^7/gm (Table 17). The arithmetic mean was 1.8×10^6/gm, the geometric mean was 1.09×10^5/gm. The highest frequency distribution was registered in range from 10^2 to 10^5/gm (72.5%). The counts in Kareish cheese were a little bit higher. The arithmetic mean was 6.87×10^6/gm, the geometric

mean was 1.44×10^5/gm, the highest count was 7.8×10^8. On the other hand only 25% of the samples had counts of at least 10^6/gm (Table 18; Figures 5 and 6).

Nearly similar findings were reported by DONNELLY, et al. (1964), DEVOYOD, et al. (1968), SOMM (1970), HOLZAPFEL & MOSTERT (1973), GHAZVINIAN, et al. (1975), MARTINEZ & FERNANDEZ (1978), DANIELSSON, et al. (1980) and TODD, et al. (1981). However FARONE (1966), HEKNEBY & GONDROSEN (1981), TESONE, et al. (1981) and BRODSKY (1984) recorded lower values, while EPSOM (1964) and IENISTEA, et al. (1971) reported higher results.

In the examined yoghurt samples the counts varied from 3×10^3/gm to 2.2×10^6/gm (Table 17); 76.6% of the samples had counts in the range $10^2 - 10^4$/gm (Table 18; Figures 5 and 6). The maximum count was 2.2×10^6/gm, the minimum 3×10^3/gm.

The recorded results were comparatively simulate those obtained by CLARK & NELSON (1961), SHARPE, et al. (1965) and ABO-EL-NAGA (1972), while LENGAUER & STUMTNER (1974), SINGH, et al. (1980) and CHUBB, et al. (1985) reported lower results.

Table (19) reveals that the F.cal. was higher than F.tab. (F.ratio at 0.05 & 0.01) so the quantitative relationship between the salt % and staphylococci content in examined Damietta cheese samples is significant and not attributed to accidentality. On the other hand table (20) shows that the F.cal. was less than F.tab. (F.ratio at 0.05 & 0.01) so

the quantitative relationship between the salt % and staphylococci content in examined Kareish cheese samples is not significant and may be attributed to accidentality. From table (21) it can be seen that the F.cal. was higher than F.tab. (F.ratio at 0.05) so the quantitative relationship between the acidity % and staphylococci content in examined yoghurt samples is significant but not due to accidentality.

36 (28.8%) of the 125 presumptive S.aureus strains proved to be S.aureus (Table 22). There were considerable differences depending on the origin of the strains (milk: 45.7%; yoghurt: 34.8%; Damietta cheese: 23.3%; Kareish cheese: 13.5%). All but 2 S.aureus strains were positive in the coagulase-test, the slide agglutination technique, Staphyslide test and termonuclease test. The two mentioned strains isolated from yoghurt samples were negative in the slide agglutination technique.

Nearly similar findings in examined milk samples were reported by THATCHER, et al. (1956), SVESHNIKOVA, et al. (1978) and AHMED (1980); while ABO-EL-NAGA (1972); P.H.L.S. (1972) and MOUSTAFA, et al. (1975) recorded lower values. On the other hand higher results were obtained by CLARK & NELSON (1961). The results recorded in examined Damietta cheese samples were in agreement to a certain extent with those obtained by DONNELLY, et al. (1964), IENISTEA, et al. (1971) and AHMED (1980). From other hand HENDRICKS, et al. (1959), ALLEN & STOVALL (1960), HAUSLER, et al. (1960), MICKELSEN, et al. (1961), RIVAS, et al. (1965) and GHAZVINIAN,

et al. (1975) recorded higher values; while GHONEIM (1963), MICKELSEN, et al. (1963), ABD-EL-RAHMAN (1972), P.H.L.S. (1972), MALESZEWSKI, et al. (1976), EL-BASSIONY (1977), SHELAIH (1979) and FEDER (1981) reported lower values. For the examined Kareish cheese samples the obtained values were comparatively simula those recorded by GHONEIM (1963), MICKELSEN, et al. (1963), ABD-EL-RAHMAN (1972), P.H.L.S. (1972) and EL-BASSIONY (1977); but ALLEN & STOVALL (1960), DONNELLY, et al. (1964), RIVAS, et al. (1965), IENISTEA, et al.(1971), GHAZVINIAN, et al. (1975) and AHMED (1980) reported higher values; while MOURSY & NASR (1964), MALESZEWSKI, et al.(1976), SHELAIH (1979) and FEDER (1981) recorded lower values. In examined yoghurt samples the reported results were higher than those obtained by P.H.L.S. (1972) and AHMED (1980).

All 36 S.aureus strains were also positive in the thermonucleaseseroinhibitions-test (Table 23). BECKER, et al. (1984) reported similar results.

In the Deoxyribonuclease plate culture test the diameters of the reaction zones of the strains tested were always equal to or larger than four times the diameters of the growth, in a few cases even larger than six times the diameters of the growth (Table 24). These positive reaction are typical for human, bovine, porcine and certain avian strains of S.aureus (DEVRIESE and VAN DE KERCKHOVE, 1979). The gained results were similar to those obtained by DEVRIESE and VAN DE KERCKHOVE (1979) and DEVRIESE and HAJEK (1980).

4 (11.1%) of the 36 S.aureus strains were enterotoxigenic, namely 2 (28.6%) of the 7 S.aureus strains isolated from Damietta cheese and 2 (40%) of the 5 strains isolated from Kareish cheese, but none of the milk and yoghurt strains. All enterotoxigenic strains produced staphylococcal enterotoxin type A. The enterotoxin types B, C_1, C_2 and D could not be detected using sac-culture technique for enterotoxin production and microslide gel double diffusion test as serological assay.

For examined milk samples recorded CECCARELLI & FRANCIA (1952), SIMKOVICOVA & GILBERT (1971), PAYNE & WOOD (1974), MAYER (1975), NISKANEN & KOIRANEN (1977), PARK, et al. (1978) and AHMED (1980) enterotoxigenic strains of S.aureus, while PAYNE & WOOD (1974) and PARK, et al. (1978) reported enterotoxigenic strains for yoghurt samples.

In examined Damietta and Kareish cheese samples the obtained values were comparatively higher than those recorded by DONNELLY, et al. (1967) and PARK, et al. (1978); while SIMKOVICOVA & GILBERT (1971), PAYNE & WOOD (1974), NISKANEN & KOIRANEN (1977), AHMED (1980) and TODD, et al. (1981) reported higher values.

Staphylococci especially S.aureus are important pathogenic organisms and cause many human infections. From other hand several outbreaks of staphylococcal food poisoning attributed to dairy products have been reported. These are characterized by sudden onset, nausea, abdominal pain, vomiting, diarrhea, exhaustion and prompt recovery. The high incidence of staphylococcal mastitis in dairy cattle

makes raw milk as well as Damietta and Kareish cheeses, which are traditionally prepared from raw milk without addition of lactic culture or colorant and can be consumed fresh, a possible disseminator of pathogenic staphylococci. On other hand the persons who manually handle cheese as well as yoghurt possibly may be carriers of coagulase-positive staphylococci, which are responsible for approximately 20% of the foodborne illnesses of established etiology that have been reported in the United States during recent year (CENTER FOR DISEASE CONTROL). The determination of S. aureus populations should provide an indicator of the likely presence of enterotoxin and food safety. Because counts of S.aureus are known to decline in foods during production and/or storage, the detection of staphylococcal thermostable nuclease (TNase) in food can serve as an indication of substantial growth of S.aureus and possible presence of staphylococcal enterotoxins as well as a public health view-point. Since milk and milk products especially Damietta and Kareish cheeses which are two of the most popular varieties of cheese in Egypt, may be a major cause of staphylococcal food borne disease in Egypt. The studying of this organism is very important from a public health view-point.

Sodium chloride content of cheese samples and acidity of yoghurt samples:

As can be seen from Table 25 the average sodium chloride content of Damietta cheese was 6.52%, that of Kareish cheese 2.95%. On the other hand the maxima were nearly equal for

Damietta cheese (9.23%) and Karelsh cheese (9.3%).
The yoghurt samples had a mean acidity of 3.71%, the minimum and maximum were 0.48% and 14.5% respectively. The question of relationships between sodium chloride content or acidity of the samples and the microbiological criteria have already been discussed.

Presence of S.aureus and TNase activity in sheeps cheese:

The 10 random samples of Bulgarian sheep cheese bought in Munich contained 10^{1} S.aureus/gm at the most, whereas the 10 Turkish sheep cheese samples had counts in the range from 4×10^{1} to 5.3×10^{3}/gm. The geometric means of the S.aureus counts were 1.6×10^{0}/gm (Bulgarian products) and 5.5×10^{2}/gm (Turkish products), the corresponding arithmetic means were 2×10^{0}/gm and 1.46×10^{3}/gm respectively.

Results obtained from examined Bulgarian cheese samples were lower than those recorded by HOLZAPFEL & MOSTERT (1973), GHAZVINIAN, et al. (1975), MARTINEZ & FERNANDEZ (1978), TESONE, et al. (1981), TODD, et al. (1981) and BRODSKY (1984). But for examined Turkish cheese samples the recorded values were in agreement to a certain extent with those obtained by TESONE, et al. (1981) and BRODSKY (1984)., while EPSOM (1964),DEVOYOD, et al. (1968), SOMM (1970), IENISTEA, et al. (1971), HOLZAPFEL & MOSTERT (1973), GHAZVINIAN, et al. (1975), MARTINEZ & FERNANDEZ (1978), DANIELSSON, et al. (1980), HEKNEBY & GONDROSEN (1981) and TODD, et al. (1981) recorded higher results.

Thermonuclease activity couldn't be detected in any of

the 20 examined sheep cheese samples. These results were not unexpected on account of the relatively low Staphylococcus aureus counts of the products.

CONCLUSIONS

The high coliform, enterococci and staphylococci counts as well as incidence of E.coli type I, Ent.faecalis in examined samples and to certain extent the presence of coagulase, TNase-positive S.aureus in raw milk & yoghurt and the occurrence of enterotoxigenic staphylococci in Damietta and Kareish cheeses indicate, that production, handling and processing of examined samples of milk and dairy products are performed under sanitary neglected measures.

The Bulgarian and Turkish sheep milk soft cheese samples drown in Munich showed low incidence of S.aureus.

The results of the bacteriological examination of examined samples make it increasingly necessary for concerned authority to impose specific standards and control measures to govern production & handling of milk as well as processing of Damietta & Kareish cheeses and yoghurt (zabady).

The following recommendation should be taken in consideration to improve the quality of produced milk and dairy products in Egypt:

1. Strict hygienic measures should be imposed during milk production, handling and cheese & yoghurt processing to prevent such contamination.
2. Heating of milk to destroy pathogenic microorganisms and rapidly cooling and continuous refrigeration of milk from time of milking until its use in making

dairy products (i.e cheese & yoghurt).

3. Prevention of recontamination of heated milk or cheese and yoghurt.
4. Periodical inspection of dairy farms as well as of dairy plants by specialists.
5. Educational programmes to those sharing in milk production & handling as well as in processing of dairy products by specialists, should be encouraged.
6. Periodical examination of persons who are in contact with milk and milk products (health certificate).

SUMMARY

I. 150 random samples of milk and milk products (40 milk samples, 40 Damietta cheese samples, 40 Kareish cheese samples, 30 yoghurt samples) were collected from different localities in Behera province. Moreover all samples were examined for the presence of coliforms, enterococci and Staphylococcus aureus, the cheese samples for their sodium chloride content and the yoghurt samples for their acidity.

The milk samples showed the following geometric mean values: 5.61×10^3 coliforms/ml, 8.44×10^3 enterococci/ml and 1.29×10^5 Staphylococcus aureus/ml. In a few samples coliforms (< 3/ml) and enterococci (< 10/ml) could not be found, Staphylococcus aureus were present in all samples (minimum: 1.2×10^3/ml).

The results for coliforms (1.85×10^3/gm) and Staphylococcus aureus (1.09×10^5/gm) in Damietta cheese were similar to those in milk, the geometric mean for enterococci, however, was higher (1.18×10^5/gm). In 20% of the samples coliforms could not be detected (< 3/gm), but all samples contained enterococci (minimum: 1×10^3/gm) and Staphylococcus aureus (minimum: 8×10^2/gm).

The microbiological quality of the Kareish cheese samples was the most unfavourable of the products tested. The geometric means run to 7.53×10^5 coliforms/gm, 2.29×10^6 enterococci/gm and 1.44×10^5 Staphylococcus aureus/gm. Enterococci (minimum: 7×10^4/gm) and Staphylococcus aureus

(minimum: 8×10^2/gm) were observed in all samples, coliforms in all but one sample.

The yoghurt samples showed the lowest geometric mean for Staphylococcus aureus (3.69×10^4/gm), whereas the corresponding values for coliforms (1.15×10^5/gm) and enterococci (3.6×10^4/gm) were higher than those registered in the milk samples. The minimum counts were: 4×10^3 coliforms/gm, 4×10^2 enterococci/gm and 3×10^3 Staphylococcus aureus/gm.

The Damietta cheese samples had a considerable higher sodium chloride content (mean: 6.52%) than the Kareish cheese samples (mean: 2.95%). The yoghurt samples had an average acidity of 3.71%.

The differentiation of the "thermotrophic" coliforms isolated showed that 87 (75.7%) of the 115 cultures were Escherichia coli type I (milk: 74.2%, Damietta cheese: 66.7%, Kareish cheese: 79.5%, yoghurt: 77.8%).

90 (69.8%) of 129 enterococci strains isolated from the tested products proved to be Enterococcus faecalis (milk: 67.7%, Damietta cheese: 64.9%, Kareish cheese: 67.7%, yoghurt: 81.5%).

10 (7.75%) of the enterococci strains produced thermonuclease, but were negative in the Staphylococcus aureus thermonuclease-seroinhibitiontest. These strains belonged to the species Enterococcus faecalis and were isolated from milk.

Only 36 (28.8%) of the 125 Staphylococcus aureus cultures (black colonies with halo on the Baird-Parker-Agar) proved to be Staphylococcus aureus (coagulase-, thermonuclease-, thermonuclease-seroinhibition-test-positive staphylococci). Significant differences existed depending on the origin of the strains (milk: 45.7% of the strains were confirmed as Staphylococcus aureus; yoghurt: 34.8%; Damietta cheese: 23.3%; Kareish cheese: 13.5%).

All Staphylococcus aureus strains showed strong DNase effects in the Deoxyribonuclease plate culture test.

4 (11.1%) of the Staphylococcus aureus strains were enterotoxigenic. These strains produced only enterotoxin type A. Two strains were isolated from Damietta cheese (28.6% of the strains) and two from Kareish cheese (40% of the strains).

II. 10 random samples each of Bulgarian and Turkish milk sheep soft cheese were collected from the market in Munich (Federal Republic of Germany) and examined for the presence of Staphylococcus aureus and thermonuclease activity.

The Bulgarian products contained 10^1 Staphylococcus aureus/gm at the most, the Turkish products between 4×10^1 and 5.3×10^3/gm. Thermonuclease activity could not be detected in any of the 20 samples.

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Media and Reagents

1-Alcoholic Naphtol Anilin Derviatives in dephenol catalest 1 %:(Merck Art.Nr.3067).

2-Baird-Parker:(Oxoid Code CM 275)

3-Barritt reagent:(Merck Art.Nr.5712).

4-Benzidine dihydrochloride solution.

5-Brain Heart Infusion Broth:(Oxoid Code CM 225).

6-Brain Heart Infusion Broth with 6.5 % Nacl.

7-Brilliant Green Bile (2 %) Broth:(Oxoid Code CM 31).

8-Bromothymol Blue Agar Base:(Hugh and Leifson, 1953).

9-Citrate Azide Agar:(A.P.H.A, 1972).

10-Columbia Blood Agar Base:(Oxoid Code CM 331).

11-Coomassie-Brilliant-Blue Solution:(Sommerfeld and Terplan, 1975).

12-DNase Agar:(Oxoid Code CM 321).

13-Double Strength Brain Heart Infusion Broth.

14-EC Broth:(ICMSF, 1978).

15-ESD:(Efthymiou, et al. 1974).

16-H_2O_2 3 %.

17-H_2O_2 5 %.

18-Kanamycin Aesculin Azide Agar:(Oxoid Code CM 481).

19-Kovacs' reagent:(Merck Art.Nr.9293).

20-Lauryl Tryptose Broth(Lauryl Sulphate Broth):(Oxoid Code CM 451).

21-Liquid Gel Solution:(Sommerfeld and Terplan, 1975).

22-Mannitol Salt Agar:(Oxoid Code CM 85).

23-McConkey Agar:(Oxoid Code CM 115).

24-MR-VP Broth:(Merck Art.Nr.5712).

25-Methyl Red Indicator solution:(Merck Art.Nr.5712).

26-Nacl 10 %:(Bailey and Scott, 1974).

27-Nutrient Broth No.2:(Oxoid Code CM 67).

28-Phosphate-Buffer Saline solution.

29-Plate Count Agar:(Oxoid Code CM 325).

30-Potassium hydroxide 3 %.

31-Potassium hydroxide 40 %.

32-Potassium-Tellurite 0.04 %:(Skadhauge, 1950).

33-Purple Broth Base:(Difco).

34-Semi-Solid Agar.

35-SIM:(Oxoid Code CM 435).

36-Simmons - Citrate-Agar:(Merck Art. Nr. 2501).

37-Stain removal solution:(Sommerfeld and Terplan, 1975).

38-SF Broth:(Hajna and Perry, 1943).

39-Toulidin blue-O-DNase Agar:(Lachica, et al. 1971).

40-3 m Trichloroacetic acid solution (TCA).

41-2, 3, 5-Triphenyl Tetrazolium Chloride 0.01 %:(Facklam, 1973).

42-0.05 m Tris-Buffer.

43-Tryptone water:(Oxoid Code CM 87).

44-Violet Red Bile Agar:(Oxoid Code CM 107).

Arabic Summary

" " بسم الله الرحمن الرحيم " "

"المبينات البكتيرية كمؤشر للنوعية الصحية للألبان وبعض منتجاته "

ملخص الرسالة

يعتبر اللبن ومنتجاته مثل الجبن الدمياطى والجبن القريش والزبادى فى مصر منذ سنوات عديدة من الأغذية الهامة التى يحتاج اليها الفرد فى جميع الأعمار • ويعتبر اللبن وجبة غذائية كاملة لأحتوائه على أهم العناصر الغذائية (البروتين الحيوانى والدهن) الى جانب احتوائه على معظم العناصر الغذائية اللازمة لبناء الجسم وخصوصا الفيتامينات والأملاح هذا بالاضافة الى سهولة هضمه وامتصاصه • وكذلك يعتبر الجبن الدمياطى والقريش من أهم المواد الغذائية التى يحتاج اليها الأنسان فى مصر كل يوم كبروتين حيوانى وتعتبر هذه المنتجات فى الدول الفقيرة مصدر غذائى كبير يعتمد عليه كل الافراد • وبالنسبة لمصر يعتمد بعض الناس محدودى الدخل على هذا المنتج اعتمادا كبيرا وخاصة فى القرى والمدن الصغيرة ولذا يجب الاهتمام بانتاج اللبن ومنتجاته من الناحية الصحية حيث يتسبب عن تناولهم الكثير من الأمراض وأهمها مرض الاسهال الصيفى فى الأطفال والذى يسببه ميكروب القولون العصوى والذى يعتبر كمبينات بكتيرية والتسمم الغذائى والذى يسببه الميكروب المكور العنقودى الذهبى والذى يكون مصدره غالبا الانسان •

ومن أهم منتجات الألبان أيضا فى مصر منتج الزبادى الذى يعتمد عليه معظم الأفراد كغذاء وترجع أهمية الزبادى الى أنه يستخدم كعلاج فى بعض الأمراض المعوية والذى يقوم بوصفه بعض الأطباء •

ويعتبر اللبن ومنتجاته من اكثر المواد الغذائية عرضه للتلوث بالعديد من الميكروبات سواء من الحيوان أو أثناء انتاجه وتداوله مما يجعله مصدرا لعدوى المستهلك لكثير من الأمراض والأوبئه وخاصه اللبن الخام والجبن القريش والدمياطى • لذلك كان من الأهمية والضرورة القيام بهذه الدراسة لتقرير الحالة الصحية للألبان الخام المتداوله والجبن الدمياطى والقريش والزبادى التى تعتبر من اكثر منتجات الألبان استهلاكا فى جمهورية مصر العربية •

وقد شملت الدراسة فحص ١٥٠ عينة من الألبان الخام والجبن الدمياطى والقريش والزبادى جمعت عشوائيا من اماكن ومصادر متعددة فى محافظة البحيرة بالأضافة الى ٢٠ عينة جبن

منتجه من البان الاغنام جمعت أيضا عشوائيا فى مدينة ميونخ بجمهورية المانيا الاتحادية على النحو التالى :—

نوع الغذاء	عدد العينات	المصدر
البان خام	٤٠	مزارع ومحلات الألبان
جبن دمياطى	٤٠	المحلات العامة
جبن قريش	٤٠	المحلات العامه والباعة الجائلين
زبادى	٣٠	المحلات العامة
جبن بلغارى	١٠	السوبر ماركت
جبن تركى	١٠	السوبر ماركت

وقد فحصت تلك العينات بكتريولوجيا وتم الفحص المبدئ فى كلية الطب البيطرى — جامعة الاسكندرية بجمهورية مصر العربية للتعرف على مدى وجود هذه الميكروبات البكتيرية والتى تعتبر كمؤشر للنوعية الصحية ، واستكمل الفحص النهائى للعينات المصرية فى كلية الطب البيطرى — جامعة ميونخ بجمهورية المانيا الاتحادية كذلك تم فحص عينات كل من الجبن البلغارى والتركى فى مدينة ميونخ وشمل الفحص ما يلى :—

الفحص البكتريولوجى :—

— التعرف على مدى تواجد ميكروب القولون العصوى Coliform count (MPN/1 ml. or gm).

— التعرف على مدى تواجد الميكروب المكور السبحى المعوى Enterococcus count/ 1 ml. or gm.

— التعرف على مدى تواجد الميكروب العنقودى الذهبى S. aureus count/ 1 ml. or gm.

وتم عزل هذه الميكروبات على أنواع المستنبتات المختلفه كما اجريت عملية التصنيف فى كلية الطب البيطرى بميونخ وكذلك التعرف على ما تفرزه من سميات وعددها كما يلى :

النوع		العدد
عترات من الميكروب القولونى العصوى	(Thermotrophic)	١١٥
" " " المكور السبحى المعوى	Enterococcus	١٢٩
" " " المكور العنقودى الذهبى	S. aureus	١٢٥

وقد تم تحديد تركيز ملح الطعام فى الجبن الدمياطى والقريش كذلك نسبة الحموضة فى الزبادى . كما تم فحص عينات الجبن البلغارى والتركى بكتريولوجيا للتعرف على مدى تواجد

الميكروب المكور العنقودى الذهبى S. aureus count/1 ml. or gm.
وكذلك الكشف عن انزيم TNase فى هذه العينات ٠

وقد تبين من الفحص أن المتوسط الحسابى لعدد ميكروبات القولون العصوية والميكروبات المكورة السبحية المعوية (المبينات البكتيرية) والميكروبات المكورة العنقودية الذهبية فى العينات التى تم فحصها كما هو مبين فى الجدول التالى :—

نوع الغذاء	ميكروبات القولون العصوية / ١ سم٣ أو جم ٠	الميكروبات المكورة السبحية المعوية	الميكروبات المكورة العنقودية الذهبية
الألبان الخام	٤٫٠٥ × ١٠^٦	١٫٥٩ × ١٠^٦	١٫٣٩ × ١٠^٦
الجبن الدمياطى	٤٫٧١ × ١٠^٦	٩٫٥٦ × ١٠^٦	١٫٨ × ١٠^٦
الجبن القريش	١٫٥٧ × ١٠^٧	٨٫٦١ × ١٠^٦	٦٫٨٧ × ١٠^٦
الزبادى	٤٫٣٨ × ١٠^٦	٢٫١٤ × ١٠^٥	٣٫٠٤ × ١٠^٥
الجبن البلغارى	—	—	١٠^١
الجبن التركى	—	—	٤ × ١٠^١ — ٥٫٣ × ١٠^٣

وقد أمكن تصنيف عترات ميكروب القولون العصوى Thermotrophic والذى يعتبر كمؤشر للنوعية الصحية المعزوله والتى تم تصنيفها فى كلية الطب البيطرى بميونخ كالآتى E. coli Type I ٨٧ (٧٥٫٧ %) من ١١٥ عترة كانت ايشريشيا كولاى من النوع الأول وكانت بنسبة ٧٤٫٢% ، ٦٦٫٧% ، ٧٩٫٥% ، ٧٧٫٨% فى عينات الألبان الخام ، الجبن الدمياطى ، الجبن القريش والزبادى على التوالى ٠ وبالنسبه للميكروب المكور السبحى المعوى والذى يعتبر من أدق المبينات البكتيرية كمؤشر للنوعية الصحية دلت النتائج أن ٩٠ (٦٩٫٨%) من ١٢٩ من العترات التى تم عزلها بمصر كانت Ent. faecalis وبنسبة ٦٧٫٧% ، ٦٤٫٩% ، ٦٧٫٧% ، ٨١٫٥% فى عينات الألبان الخام ، الجبن الدمياطى ، الجبن القريش والزبادى على التوالى ٠ ومن جهة اخرى وجد أن ١٠ (٧٫٧٥%) من عترات الميكروب المكور السبحى المعوى تفرز انزيم TNase ولكن كانت النتائج سلبية بالنسبة لاختبار S. aureus thermonuclease-seroinhibition test.
ومن ١٢٥ من عترات الميكروب المكور العنقودى الذهبى والتى ظهرت على مستنبت

Baird-Parker Agar باللون الأسود وتحدها من الخارج طبقة خفيفة باهته وجد أن ٣٦ فقط (٢٨ر٨ ٪) كانت S.aureus وبنسبة مختلفة ومعتمده على نوع الغذاء التى عزل منه العترات كالآتى ٤٥ر٧ ٪ ، ٣٤ر٨ ٪ ، ٢٣ر٣ ٪ ، ١٣ر٥٥ ٪ فى عينات الألبان الخام والزبادى والجبن الدمياطى والجبن القريش على التوالى . وقد لوحظ أن كل عترات S.aureus لها تأثير قوى لانزيم DNase على DNase plate culture test. وقد أثبت أن ٤ (١١ر١ ٪) من عترات S.aureus كانت من النوع المفرز للسموم وأثبت أن كل العترات تفرز فقط نوع السم أ وكانت عدد ٢ عترة فى كل من عينات الجبن الدمياطى (٢٨ر٦ ٪) والجبن القريش (٤٠ ٪) .

وتم تحديد نسبة تركيز ملح الطعام فى كل من عينات الجبن الدمياطى والجبن القريش وكان المتوسط الحسابى هو ٦ر٥٢ ٪ ، ٢ر٩٥ ٪ على التوالى ، وأما بالنسبه للحموضة فكانت ٣ر٧١ ٪ فى عينات الزبادى .

وفى كلية الطب البيطرى بميونخ بجمهورية المانيا الاتحادية تم فحص ١٠ عينات جمعت من محلات السوبر ماركت من كل من جبن الاغنام البلغارى وجبن الأغنام التركى للتعرف على مدى تواجد الميكروب المكور العنقودى الذهبى S.aureus ونشاط انزيم TNase وقد وجد من الفحص أن العدد الكلى للمكورات العنقودية الذهبية S.aureus كانت 10^{1} ، بين ٤ × 10^{1} ، ٣ر٥ × 10^{3} لكل ١ جم من الجبن البلغارى والتركى على التوالى كما هو مبين بالجدول السابق ولم يثبت نشاط انزيم TNase فى جميع العينات التى تم فحصها .

وتدل زيادة عدد الميكروبات المختلفة سالفة الذكر فى عينات الألبان الخام ، الجبن الدمياطى ، الجبن القريش والزبادى ، ووجود ميكروبات Ent.faecalis, E.coli Type I فى العينات التى تم فحصها والتى تعتبر كمبينات بكتيرية ومؤشر للنوعية الصحية للألبان ومنتجاتها والى حد ما وجود Coagulase, TNase + ve S.aureus فى اللبن الخام والزبادى ووجود العترات التى تحتوى على السم أ فى الجبن الدمياطى والقريش بدون شك أن انتاج وتداول الألبان الخام ومنتجاتها كالجبن الدمياطى والقريش والزبادى فى العينات التى تم فحصها فى حالة سيئة وغير مرضية .

وانتهى البحث الى اصدار التوصيات الآتيـــة من أجل انتاج غذاء صحى وسليـــم : —

١) ضرورة توافر الاشتراطات والنواحى الصحية السليمة فى انتاج وتداول هذه الألبـــان الخام ومنتجاتها لمنع التلـــوث .

٢) معاملة الألبان بالحرارة اللازمة لقتل الميكروبات المرضية ثم سرعة تبريده وحفظه فـــى الثلاجات من فترة انتاجه وحتى استخدامه فى تصنيع الجبن والزبادى .

٣) منع تلوث اللبن المسخن أو منتجاته مثل الجبن والزبادى مرة أخـــرى .

٤) عمل فحص دورى بواسطة الأخصائيين على مزارع ومصانع الألبان ومنتجاتهـــا .

٥) عمل برامج تعليميـــة ارشادية للعاملين فى انتاج وتداول الألبان وكذلك تصنيع منتجاتها بواسطة اخصائيين والعمل على تشجيع هذه البرامج .

٦) عمل فحص صحى دورى للعاملين فى مجال انتـاج الألبـــان وتصنيعـــه .

٧) عمل مقاييس خاصة للحكم على انتاج وتداول الألبان ومنتجاتها فى جمهورية مصر العربيـة .

تحت اشـــــــــــــراف

الاســـــتاذ الدكتـــــور

عبـــــاس أمين احمـــــــــــــــــــــد

استاذ الرقابـــة الصحيـــه علي الالبـــــــــــان ومنتجـــاتها

رئيس قسم الرقابه الصحيه علي الاغذيـــة ووكيل كليه الطب البيـــــطري

جامعـــة الاســـــكندرية

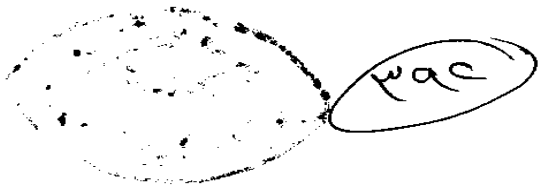
دكتـــــــــــــور

ك ٠ زاد هـــــــــــــــــــــــــــوف

المباشـــــر الاكـــــــاديمـــــــــــــــي

كليه الطـــــــب البيـــــــــــــــطري

جامعـــــة ميونـــــــخ



المبينات البكتيرية كمؤشر للنوعية الصحية للألبان وبعض منتجاته

رسالة مقدمة من

السيد ط . ب / عادل مصطفى الخولى

بكالوريوس فى العلوم الطبية البيطرية ـ جامعة اسيوط ـ ١٩٧٨

ماجستير فى العلوم الطبية البيطرية ـ تخصص رقابة صحية على الألبان

ومنتجاتها ١٩٨١ ـ جامعة الأسكندرية

للحصول على

درجة الدكتوراه فى فلسفة العلوم الطبية البيطرية

الرقابة الصحية على الألبان ومنتجاتها

الى

كلية الطب البيطرى ـ جامعة الاسكندرية

١٩٨٦