Bacterial quality of three casserole dishes served in a university food service
by Mary Eleanor Ginther

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Home Economics
Montana State University

Abstract:
Bacteria may be transmitted through foods and cause discomforting gastrointestinal, illnesses in unsuspecting persons who ingest such contaminated foods. Particulary prevalent pathogens of concern to manufacturers, processors, and consumers of food are the organisms - Staphylococcus aureus, Salmonella, and Clostridium perfririgens. The ability of these bacteria to survive -and proliferate in foods depends on several factors such as pH, water activity, mechanical forces, ingredients, competitive saprophytic organisms and temperature. The latter factor is probably a major one in large quantity cookery but total bacterial response will determine if the food will exhibit an antagonistic or beneficial effect on the exogenous organisms.

The current study was done to evaluate three casseroles bacteriologically which were processed and served from one dormitory cafeteria on the campus of Montana State University. The food services had recently initiated an expanded program of serving which necessitates foods to be maintained at warming temperatures for longer periods, which could be hazardous if not carefully controlled. The methods used in this study are those commonly used and accepted by authorities in food microbiology. The results show that the foods tested were of. high bacteriological quality.

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Date August 9, 1972

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A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree

of

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Home Economics

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August, 1972

ACKNOWLEDGEMENTS

I would like to express due appreciation to those persons who directly supervised me in this task as well as to parents, sister and numerous friends who lent support as well as challenge to me in graduate school.

My thanks and gratitude to:

Dr. Andrea Pagenkopf who donated constant effort and much extra time as well as much patience and guidance as a friend and advisor.

Mr. Glen Lewis, as well as other supervisors and employees of the Campus Food Service, whose cooperation made this study possible.

The members of my committee who were very helpful and cooperative in their efforts. This includes Dr. John Jutila, Dr. Robert Lind, Dr. Robert Dynes, and previously Dr. James Boyd.

A very special thanks to my parents, my sister Sonja, and Mrs. Karen Olsen who aided me in numerous tangible and intangible ways, and who were so consistent in this help throughout my entire program of study.

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ABSTRACT

Bacteria may be transmitted through foods and cause discomforting gastrointestinal illnesses in unsuspecting persons who ingest such contaminated foods. Particulary prevalent pathogens of concern to manufacturers, processors, and consumers of food are the organisms - *Staphylococcus aureus*, *Salmonella*, and *Clostridium perfringens*. The ability of these bacteria to survive and proliferate in foods depends on several factors such as pH, water activity, mechanical forces, ingredients, competitive saprophytic organisms and temperature. The latter factor is probably a major one in large quantity cookery but total bacterial response will determine if the food will exhibit an antagonistic or beneficial effect on the exogenous organisms.

The current study was done to evaluate three casseroles bacteriologically which were processed and served from one dormitory cafeteria on the campus of Montana State University. The food services had recently initiated an expanded program of serving which necessitates foods to be maintained at warming temperatures for longer periods, which could be hazardous if not carefully controlled. The methods used in this study are those commonly used and accepted by authorities in food microbiology. The results show that the foods tested were of high bacteriological quality.

CHAPTER I

INTRODUCTION

Certain microorganisms and parasites are transmitted through foods and may cause illness to persons who ingest these foods. For some microorganisms food is merely a vehicle of transmission. For others it is a good medium of growth. Outbreaks of acute intestinal enteritis caused by the ingestion of a food in which pathogens have multiplied are referred to as food poisoning outbreaks. Microorganisms which may cause foodborne illness include bacteria, viruses, rickettsiae, and protozoa. Bacteria are the organisms which most commonly cause gastrointestinal illness. Common symptoms are intestinal upset, vomiting, diarrhea, nausea, and malaise (1).

Each year thousands of cases of food poisoning are reported in the United States. These reports vary widely from state to state. There is reason to believe that the number of foodborne illnesses is much higher than the reported statistics show. Reporting is not compulsory in any state, and it is false to assume that the states with the greatest number of cases have the poorest sanitary conditions. Very often small outbreaks, say in a family, go unreported while the more dramatic outbreaks involving hundreds of people receive undue attention. Contrastingly, large outbreaks may receive no attention at all due to an organization's fear of publicity. Needless outbreaks continue with the accompanying economic losses and the manifold problems of resulting illnesses. Proper surveying of outbreaks could increase the knowledge of foodborne diseases. Reliable information is needed in order to protect the consumer.

Occurrences of foodborne illnesses have been reported from many types of food service establishments--restaurants, hospitals, and schools, for example. Paramount to an effectual sanitary control of menu items is an understanding of the reservoirs of the potentially dangerous organisms, conditions favoring food contamination in food preparation areas, and the conditions allowing profuse growth of bacteria in foods. Some foods are much more likely to be indicted in occurrence of foodborne illness due to the nature of their ingredients and the handling procedures they receive. The food handlers practice effective sanitary habits, use fresh and unadulterated ingredients, and allow foods to undergo adequate heating or cooling. Prepared foods, such as casseroles or sandwich spreads, are thus a good indication of the adequacy of all the foregoing factors.

Keeping the above points in mind, a study was undertaken to evaluate the bacteriological quality of certain prepared foods in a college cafeteria. Casseroles were chosen because they represent a major menu entree which is frequently offered. Also, such dishes are a potpourri of ingredients--fresh foods, and leftovers, such bacteriologically susceptible items as milk and eggs, and also some less vulnerable items like noodles. These casseroles contain major ingredients such as: beef, pork, poultry, or fish--which is surrounded by a starchy base. The total result is a protein and carbohydrate mass which is readily susceptible to bacterial contamination (2). Cooking procedures should render such dishes safe from harmful pathogens. There is an additional danger in cafeterias or other public eating places where food must be maintained at temperatures hot enough to stop bacterial proliferation, while not so hot that the aesthetic appeal of the food is destroyed. The task of the food service is to take raw ingredients and formulate them into a safe and attractive food dish. The final goal is to heat the food enough to eliminate pathogens and maintain it at temperatures which keep the food continually safe for the consumer.

The present study was undertaken for three major reasons, which are as follows: the large percent of outbreaks occurring in schools, the current extended serving hours now used in many cafeterias, and the lack of data in this area. Recently, many college cafeterias have been experimenting with using an increased number of hours during which food is served during the day, in order to accommodate students more amply. It is not improbable to think that such extended serving hours might increase the susceptibility of food to bacterial contamination. In 1968, 1969, and 1970, the incidences of food poisoning in various public food establishemts was 44%, 30%, and 32% respectively of all places where such illnesses occurred. In 1969, 70% of these occurrences were in schools (3, 4). These factors lend some probability to the premise that such dormitory food could be a good reservoir for pathogens since these foods are kept at holding temperatures for such long periods of time. The information inherent in such a study would be of interest to food service managers. It would allow them to know more accurately the bacteriological quality of the food served to the customers.

Hypotheses

1. Is there any evidence of _Clostridium perfringens_, _Salmonella_, or _Staphylococcus aureus_ in the casseroles examined bacteriologically in this cafeteria?

2. If there is evidence of contamination, would proper heat treatment have eliminated the danger?

3. Are increased sanitation procedures needed to insure a greater amount of consumer safety? If so, what reliable suggestions can be made?

4. Do the lengthened serving hours have implications as far as food safety in this cafeteria is concerned?

CHAPTER II

REVIEW OF LITERATURE

Introduction

Disease or illnesses, a state wherein people operate at a less than optimal level, is a definite concern to those interested in public health. One of these suboptimal states is bacterial illness. In particular, this paper will deal with sicknesses developing from the consumption of various contaminated foodstuffs. Several species of bacteria are exogenous contaminants of food. Some foods are conducive to the proliferation of bacterial contaminants, making these foods good vehicles for foodborne illnesses. The organisms which engender these illnesses in humans via foods are called food poisoning organisms. The resulting maladies are characterized generally by a short incubation period after the consumption of the dangerous food. Gastrointestinal upsets are the earliest symptoms accompanied later by less localized manifestations. The most frequently reported causes of food poisoning are members of the *Salmonella* species, *Staphylococcus aureus* (*Staph. aureus*), and *Clostridium perfringens* (*C. perfringens*) (3, 4).

Statistics of Foodborne Illnesses

Statistics from previous years as reported in *Foodborne Outbreaks* (3, 4) yearly summaries show that public food service establishments are the most common places of acquisition of foodborne disease. This includes restaurants, delicatessens, cafeterias and schools. Schools were responsible for 70 percent of all reported cases in 1969 and 21 percent of all persons acquiring illnesses in all places in 1970. (See Table 1 on page 8.) Evidence of the importance of Staph. aureus, Salmonella, and C. perfringens as factors in the incidence of foodborne diseases is given in Table 2, page 9. These three organisms together were responsible for 56 percent of all bacterial caused foodborne outbreaks in 1969 and 1970 (3, 4). Staphylococci account for the largest percentage of outbreaks from one bacterial species. Beef accounted for 16 percent of all vehicles in 1969, pork 10 percent turkey 8 percent. The respective figures for 1970 were 18 percent, 16 percent, and 11 percent for a total of 45 percent in 1970 and 34 percent in 1969 of all foods which harbored food poisoning organisms. Table 3, page 10, shows the three most prominent foods responsible for illnesses caused by the three types of organisms. Data available shows a higher incidence of foodborne disease in the warmer months from April to October (3,4). The variation for all months is not great.

Table 1

THE NUMBER AND PERCENT OF CASES AND OUTBREAKS OF FOODBORNE ILLNESSES ACQUIRED IN SCHOOLS AND RESTAURANTS, UNITED STATES, 1969-70[a]

	1969				1970			
	Cafeteria Restaurants		Schools		Cafeteria Restaurants		Schools	
Number of persons ill	3,904	(14%)	19,842	(70%)	9,580	(31%)	4913	(21%)
Percentage Outbreaks caused by:								
Staph. aureus	27	(25%)	5	(13%)	33	(26%)	8	(30%)
Salmonella	7	(6%)	3	(8%)	18	(15%)	3	(12%)
C. perfringens	33	(30%)	17	(45%)	27	(20%)	7	(28%)
Total caused by above	67	(61%)	25	(66%)	78	(61%)	18	(70%)
Other causes	43	(39%)	38	(34%)	51	(39%)	8	(30%)
Total	110	(100%)	63	(100%)	129	(100%)	26	(100%)

[a]United States Department of Health, Education and Welfare Foodborne Outbreaks Annual Summary. Atlanta, Georgia: Center for Disease Control. Page 16. 1969-1970.

Table 2

FOODBORNE OUTBREAKS OF BACTERIAL ORIGIN AND THE PERCENTAGE CAUSED BY STAPHYLOCOCCUS AUREUS, SALMONELLA, AND CLOSTRIDIUM PERFRINGENS, UNITED STATES, 1969-1970[a]

	% of total 1969	% of total 1970
Foodborne outbreaks of bacterial origin	66	66
Percent of Bacterial Foodborne Outbreaks Caused by:		
Staph. aureus	25	28
Salmonella	13	13
C. perfringens	18	15
Total of outbreaks caused by three organisms	56	56

[a]United States Department of Health, Education, and Welfare. Foodborne Outbreaks Annual Summary. Atlanta, Georgia: Center for Disease Control, page 3, 1969-1970.

Table 3

THREE MOST FREQUENT VEHICLES ASSOCIATED WITH FOODBORNE ILLNESSES CAUSED BY EACH OF THE ORGANISMS, STAPHYLOCOCCUS AUREUS, SALMONELLA, CLOSTRIDIUM PERFRINGENS IN THE UNITED STATES, 1969-1970[a]

Foods Incriminated by:	% of Total 1969		% of Total 1970	
Staph. aureus	Pork	30%	Pork	20%
	Beef	15%	Unknown	15%
	Turkey	11%	Bakery Products	12%
Salmonella	Turkey	20%	Unknown	20%
	Unknown	20%	Turkey	17%
	Chicken	13%	Beef	13%
C.perfringens	Beef	47%	Beef	42%
	Turkey	22%	Turkey	24%
	Vegetables, Fruit	6%	Unknown	9%

[a]United States Department of Health, Education, and Welfare. Foodborne Outbreaks Annual Summary, Atlanta, Georgia: Center for Disease Control, page 14, 1969-70.

These statistics give some indication of the prevalence of these types of bacterial food illnesses and thus the importance of preventive measures in food service establishments. This information is indicative of all reported incidences, but it should not be assumed that it is complete for all occurring cases.

Characteristics of Food Poisoning Microorganisms

Foodborne illnesses are of two types: Infections and intoxications. An infection occurs when a large number of live organisms are ingested. An intoxication, however, is the result of the ingestion of a toxin produced by an organism. Live cells need not be present or be ingested in order for an intoxication to ensue. The distinction between these two types is of significance in determining the heating procedure needed for making a product safe for consumption (1).

Salmonellae

Salmonellae are small gram negative rods, which grow very well either aerobically or anaerobically. The organisms grow optimally at room temperature. Nutritionally its requirements are not fastidious--demanding neither vitamins nor amino acids. An outstanding trait of this group is its inability to ferment lactose (5). The illness, salmonellosis, caused by any of the genus of Salmonella, is an infection caused by the ingestion of the bacteria themselves. It is usually characterized by a 6-18 hour incubation period, followed by gastrointestinal symptoms such as diarrhea, and perhaps vomiting. An elevated temperature is not uncommon.

Recovery is rapid, usually only a few days (6). The organisms are easily destroyed by temperatures high enough to pasteurize milk (79 C. for 25 seconds) (1).

Staphylococci

Staphylococcal food poisoning, which is often said to be the most common type (7), is caused by the ingestion of a toxin which may be produced by proliferating cells of Staph. aureus. The organism is a gram positive coccus commonly found in grape-like clusters. One notable characteristic is the ability of the organisms to ferment mannitol. Amino acids and several vitamins are essential to the growth of Staph. aureus (5). Millions of microorganisms must be present for adequate toxin to be produced to cause disease. Nausea, vomiting, diarrhea, malaise, and weakness occur in the ailment, the onset of which is shortly after eating--thirty minutes to three hours (6).

The prime forces in staphylococcal food poisonings are the enterotoxins. There are five types--A, B, C, D, and E--and of these types, A is implicated in most cases of illness. Several reasons have been given for this higher incidence. One is that type A is a primary metabolite while type B is produced secondarily. Also, humans seem to be more susceptible to enterotoxin A. And since pH 6-7 is preferred for production of type A, prepared dishes are a good medium since their pH is usually within this range (8). The only reliable method of determining if a food has caused staphylococcal food illness is to isolate the toxin. The presence of coagulase positive staphylococci per se is not indicative of the illness as shown by the existence of enterotoxin-forming coagulase negative staphylococci as well as coagulase positive staphylococci which produced no enterotoxin and so were safe for consumption (9). The "presence of rapid growth and high numbers of a potentially enterotoxigenic strains of Staph. aureus, though undesirable, would not necessarily indicate the presence of enterotoxin" (10). Most experts agree that staphylococci must be present in high numbers (10^6-10^8 cells per gram of menstrum) before a more than negligible amount of toxin can accrue. In raw and pasteurized milk, a 5×10^7 cell concentration per milliliter was necessary before enterotoxin A was produced (11). The toxin is very stable to high temperatures which will not inactivate it (2). This thermostability is partially due to a binding of enterotoxin to various proteins such as myosin and metmyoglobin in meat (12). The production of enterotoxin is influenced by the prevailing natural food flora (13), by aeration (14), and by pH. The production of enterotoxin A is highly sensitive to acidity and so is very unlikely to be produced in acidic foods (15).

Clostridium perfringens

C. perfringens causes a mild malaise, accompanied by abdominal pain and diarrhea with an onset of illness 8-18 hours after the ingestion of contaminated food. The factors responsible for inducing disease are not yet clearly understood. But it is known that populations of one million organisms per gram of food are required before disease results (6). The bacterium is a plump, nonmotile gram positive rod occurring singly or in chains. Capsules are usually present and will develop in the absence of essential cellular nutrients. Vegetative cells are strictly anaerobic and nutritive requirements are complex with nineteen amino acids and numerous vitamins, and minerals essential for growth. Distinct toxins are formed each having preferential conditions of incubation, medium, and pH. The alpha-toxin is the one commonly associated with the virulence of this organism (5).

The spores of C. perfringens were found to withstand internal terminal temperatures of 74 to 77 C. (2). Spore formation occurs when vegetative cells are in an environment lacking nutrients or other necessary constituents. Authorities usually recognize two types of C. perfringens--the heat resistant and the heat sensitive. The former is more common in England while the latter is more prevalent in the United States. Heat resistant types will survive temperatures of 100 C. for sixty minutes and do not form spores as readily as the heat sensitive types (16).

Factors Affecting the Growth of Microorganisms in Foods

Temperature

Heating

The major safety control mechanism used in large quantity cookery is cooking proceeded by adequate cooling of foods (2). There is still much unknown about the total effect of heat on bacterial cells. Sublethal heat can produce a variety of reparable lesions in a cell (17, 18, 19). The amount of cells which will be destroyed by heating is dependent on numerous factors including the degree and type of heat, and the status of the organisms. The type of heat has an effect on the destructability of microorganisms. Knowledge of the damage by moist heat in nonsporeforming microorganisms remains incomplete. At equivalent temperatures, moist heat is usually more lethal than dry heat to a bacterial cell--damaging the essential protein complexes. Salmonellae are highly sensitive to moist heat, and their resistance to heat increases steadily when moisture is reduced (20, 21, 22). Baldwin et al. found that S. typhi and Staph. aureus were destroyed more completely when cooked four minutes electronically than when cooked 30-40 minutes in a conventional oven (23). Walker et al. reported that older cultures of Staph. aureus required three times longer heating for destruction than twelve hour cultures (24). Thermal time considerations are complicated by the fact that some bacteria are more or less resistant to high temperatures than others. Read et al. found that the D value (time required to kill 90% of bacteria) at 68.3 C. for S. senftenberg 775W (heat resistant strain) was 10 seconds while .28-.52 seconds were required for 90% kill of the other six strains tested in whole milk (25). Although most salmonellae strains are quite heat sensitive, it has been recommended that S. senftenberg 775W be used as a reference strain because heat treatment adequate to destroy it will likely destroy all other salmonellae and staphylococci present (26, 27). Killing temperatures are also dependent on the initial bacterial load as was shown by Read and his coworkers with concentrations of S. senftenberg 775W, in which pasteurization temperatures were satisfactory unless numbers greater than 3 x 10^{12} organisms per milliliter were present (25). Thomas et al. studied Staph. aureus and S. senftenberg 775W, in four media and showed the need for establishing probable numbers in a type of food before deciding a valid heating time. Large solid particles in a food will alter heating times (26). Some researchers have suggested an internal temperature of 74 C. for eleven minutes to kill salmonellae, allowing some margin of safety (28).

Effect of Heat on Enterotoxin Production and Destruction

The presence of staphylococci in a food is not proof that enterotoxin is also present. Toxin production depends on several factors including temperature. Donnelly et al. in one study found that enterotoxin was readily produced at 35 C. The organisms were less able to produce toxin at 30 C., 25 C., or 20 C. No toxin was produced at 10 C. in either raw or pasteurized milk (13). In barbecued chicken enterotoxin was produced at 35 C. but not at 40 C., 42 C., or 45 C. (29). There is usually a delay in enterotoxin production after Staph. aureus has been in the lag phase, but this will resume as soon as favorable conditions are restored (30).

The toxin production of C. perfringens is poorly understood. There is no certainty that it is formed in food. Rather, it may be produced when vegetative cells multiply and grow in the intestine of a susceptible host (31). The toxin was found more stable and active for longer times at 100 C. than at 75 C. (32).

Destruction of toxins is another effect of elevated temperatures. Denny et al. demonstrated that the greater the toxin concentration the more heat is needed to inactivate it (33). Pure preparations of staphylotoxin were lacking in thermostability compared to crude preparations. At 121 C. the crude toxin required 1.7 times longer heating times than did pure toxin for inactivation. This is due in part to the protection provided by proteins in a menstrum (12, 34). Toxins have been found to be more stable for longer times at 100 C. in saline than in ground beef slurry. It was more quickly destroyed at 80 C. in ground beef than at 100 or 110 C. (12, 32).

Effect of Heat on Spores

Food processors should be aware of the relation of heat to spore destruction and germination. The heat needed is dependent on many factors including the food medium. In beef, spores numbering 10^5 per gram survived steaming for five hours although there was a sevenfold decrease each hour as reported by Barnes and his coworkers (35). Spore formation was maximum at pH 6 at 30 C. in a medium void of oxygen. Ahmed and Walker found that a prerequisite condition was a twenty minute period of heat activation at 75 C. (36). Vegetative cells were killed at 74 C., but increased temperatures activated spores (37). Several studies with beef showed that only 3 percent of C. perfringens spores at any temperature germinated without heat shock, thus causing raw meat to be a poor sporulation medium (35, 38).

Recovery of Microorganisms after Heat Treatment

The recovery of organisms which have experienced a damaging period of heating is an important aspect of food safety. Recovery is affected by the physiological state of the bacterium, the heating medium, and the recovery medium (39). It is generally agreed by researchers that there is an extended lag phase following sublethal heating (30, 39, 40). Jackson and Woodbrine stated that the generation time after the lag phase may remain the same as for unheated cells (30). Staph. aureus heating in 10 percent NaCl solution showed a lag time two or three times greater than unheated cells and a reduced range of temperatures in which it could repair (19). Growth was immediate on a good medium, but was somewhat slower for heated cells than for similar unheated cells (41).

The temperature of the recovery medium plays a role in the cell's ability to resume normal growth. Staph. aureus exhibited a smaller temperature growth range during revival (19). In one study Nelson showed the maximum temperatures for repair for gram positive organisms to be 32 C. at pH of 7, while a pH of 6 was more conducive to the growth of gram negative organisms (42). Salmonellae recovered rapidly at 30 to 37 C., and somewhat slower recovery occurred at 10 and 20 C. No significant growth occurred after five days at 5 C. (43).

Cooking Temperatures

Maintenance of good bacteriological quality in a finished product depends on the handling practices and proper heating of the final product. Those products not demanding testing for edibility must be properly treated. The food service establishment bears responsibility for taking nonsterile ingredients and combining them into an aesthetic and safe food product. Considering the large number of food poisoning outbreaks for which food establishments are responsible, it seems surprising that more studies have not been done on product safety and the factors that affect it

(44). Many foods depend on a period of effective heating to make them safe for the consumer to eat.

No multiplication should occur in any part of a food held at cooking temperatures. The temperature required to prevent multiplication will depend on the food and the organism. Due to certain protective or antagonistic ingredient effects and other factors, a "safe" temperature for all foods cannot be stated. Likewise, temperatures which will kill vegetative cells do not necessarily kill spores or inactivate toxins. Temperatures at or near boiling should kill salmonellae, Staph. aureus, faecal streptococci and the vegetative cells of C. perfringens (2).

Various studies have elucidated the processing temperatures adequate for making certain dishes bacteriologically safe while still maintaining the aesthetic quality. Angelotti and Foster found oven temperatures of 218 C. prevented the growth of salmonellae and staphylococci in custard, ham salad, and chicken a la king (45). All S. typhimurium were destroyed in chicken casserole held at room temperatures for four hours before cooking and then cooked at 177 C. for 40 minutes in a study by Wiedman et al. (46). C. perfringens survived temperatures of 94 C. in stuffed turkeys causing a hazard at subsequent storage temperatures which permitted multiplication (47). Hall and Angelotti found vegetative cells of C. perfringens were completely inhibited at 49 to 52 C. in meats although rapid growth occurred at 45 C. (48). Barbecued chicken had to be maintained at internal temperatures above 60 C. to destroy vegetative cells of C. perfringens (29). A study by Gunderson and Kereluk on the survival of Staph. aureus, Escherichia coli (E. coli), Streptococcus faecalis (S. faecalis), and Bacillus cereus (B. cereus) in meat pies showed survival to be a mere .0001-.1 percent after 40 minutes at an oven temperature of 217 C. Counts for all organisms continued dropping during a 10 to 20 minute waiting period after baking except for Staph. aureus, which demonstrated no change at all after removal from the oven (49). The bacteria in turkey and beef held at 68 C. during cooking were viable for six hours with no marked change in stability (50). Heating turkey and dressing to 74 C. internally was found inadequate to kill C. perfringens spores. If not served or chilled immediately the turkey and dressing were potentially dangerous (37). In studies with Staph. aureus and S. senftenberg 775W (the former is heat sensitive) with custard, ham salad and chicken a la king Angelotti et al. indicated that heating perishable foods of that type to 66 C. internally and holding every particle of food at this temperature for at least twelve minutes will reduce ten million or less salmonellae or staphylococci per gram to nondetectable levels. Equally effective was 60 C. for 78-83 minutes (45). Bayne et al. agreed that 66 C. is a high enough temperature to kill an innoculum of 2.5 x 10^8 organisms per gram of S. typhimurium and S. senftenberg 775W in chicken a la king and ground chicken meat (51). Various factors determine the effect of heat on survival of bacteria in foods (52).

Cooling Temperatures

The cooking zone is described as the temperature range which should be high enough to destroy vegetative cells in a relatively short time. Warming temperatures should utilize temperatures high enough to prevent multiplication although usually not killing microorganisms. The danger zone is that range from 7 to 60 C. in which multiplication of organisms is the greatest. The chilling zone should permit temperatures low enough to prevent multiplication over an extended storage period (2). The Public Health Department is aware of this in stating its regulations. Processed foods are safe above 60 C. and below 7 C. with hazards predictable between these temperatures (52). Such things as overcrowded refrigerators, poor cooling systems, or food aliquots too large to allow even cooling tend to make cooling a dangerous process.

The properties of a food affect the minimum temperature at which it will support bacterial growth. Most food poisoning organisms grow in a narrow pH range when at low temperatures (54). The common food poisoning organisms will not proliferate below freezing. Viable organisms decline rapidly at temperatures just below freezing (-2 C.). The decline is less rapid at temperatures lower than this (55). Plate counts done on turkey and beef held at 5 to 10 C. indicated stabilization or decrease (50). Temperatures below 6 C. are needed to prevent the growth of staphylococci and salmonellae in foods. Storage longer than one day will result in a very slow increase, unless the food is grossly contaminated. This proved true in custard, chicken a la king, and ham salad. Multiplication was slowest in custard. Toxigenic staphylococci will multiply at a temperature of 8 C. (56). Staph. aureus showed little growth in cooked ham at 4 C. (57). Lewis et al. found that a 100-serving quantity of chicken salad showed little bacterial increase after 72 hours at 10 C. (58). C. perfringens demonstrated no growth in the range of 5 to 15 C. even after five days' storage (48). The spores of C. perfringens survived six months at -5 C. and -20 C. There was little change in spore counts at temperatures of 1 C., 5 C., 10 C. or 15 C. Vegetative cells were slowly destroyed at these temperatures (35). Another researcher found a reduction of two or more logs in viable vegetative cells of C. perfringens at refrigeration or freezing temperatures (59). Of the three organisms of concern here, salmonellae are the most sensitive to freezing (55). Foods held below 5 C. will not support growth of this bacterium. Growth of salmonellae in processed foods was prevented below 5.5 C. Storage of processed foods at 10 C. will not eliminate the multiplication of dangerous organisms (54).

Holding Temperatures

It is difficult to state how many hours a food can be kept safely in the 7 to 60 C. range. There are several factors which influence holding time. It is presently agreed that the time should not exceed four hours, but that two hours would lend a better margin of safety (2, 60). Salmonellae and staphylococci did not multiply in custard held at internal temperatures of 47 to 49 C. but decreased in numbers. In chicken a la king, staphylococci grew at 44 C. but were killed at 47 C. The results of this study indicate that the range of 6.5 to 46 C. is the danger zone for bacteria growing in processed foods (60). Chicken salad that was held at room temperature (25 C.) for 24 hours exhibited a rapid bacterial increase (58). At 40 C., S. typhimurium grew, as did C. perfringens, in barbecued chicken (29). Salmonellae and staphylococci grew well in chicken a la king at 35 C. The counts decreased 60 to 90 percent in 12 to 24 hours as temperatures rose to 45 C. and 49 C. (60). Casseroles held at 20 C. to 35 C. for four hours were not harmful, but periods to eight hours were not recommended (46). Total plate counts from beef and turkey increased rapidly at 24 C. in six hours. At 37 C. cells increased rapidly in four hours (50). Rapid growth of C. perfringens cells occured at 45 C. in meat and meat dishes. Researchers advocate caution in foods cooked and held at 43 to 46 C. because C. perfringens heat resistant spores are likely to germinate (48).

Ingredients in a Recovery Medium

The type of ingredients in a food menstrum has a marked effect on the ability of cells to recover and survive after heating processes. Bacterial cells have more exacting nutrient demands following heat damage, as do spores, and are possibly more sensitive to antagonistic ingredients

(61, 62). The ingredients--as part of the total environment--help determine if a particular organism or group of organisms can withstand heat treatment in a given food. While an ingredient may exert a markedly lethal effect on a microorganism in one situation, it may be enhancing in another. Thus, ingredient effect is not a static phenomenon.

Very closely related to this is the available water (Aw) in a food. Aw is a property governed largely by the commodities (solutes) in the food which bind a certain amount of water in that food. Ingredients, in this way, also exhibit a protective or antagonistic role towards microorganisms. One study of Calhoun and Frazier revealed that the diffusion of solutes into gram positive cells was more rapid than into gram negative cells with their higher lipid content. Thusly, *Staph. aureus* was killed more promptly than *E. coli* regardless of the solute used (63). It is impossible to draw any conclusions concerning the effect of Aw because present research does not indicate that it is the sole reason for the effect of ingredients on bacteria. Rather, there is probably some overall ingredient effect (64). Aw has influence on spore production for it seems that *C. perfringens* requires a higher Aw for spore production than for vegetative growth (65). The production of enterotoxin is also influenced by Aw. When the initial number of staphylococcal cells was greater than 10^7-10^9 cells per gram, no enterotoxin was produced if the Aw was less than .96. This is partly due to the marked effect of reduced Aw on the vegetative cells of Staph. aureus (10).

Studies have been done which exhibit the protective or antagonistic effect of various food constituents on microorganisms. For example, sugars generally have a more protective effect on microorganisms than does NaCl. However, E. coli and Staph. aureus tend to have more resistance to thermal destruction when NaCl is available in comparison to sugar (20, 63). One study submitted that the medium in which salmonellae are grown has little or no influence on the heat resistance of the organisms (52). Many other researchers in the area agree that, on the contrary, most microorganisms are influenced either positively or negatively by the food components, including fats, proteins and carbohydrates (64, 66, 67, 68, 69, 70). These effects are noticeably variable.

The effect of the food components is in part due to the temperature of the menstrum. S. typhimurium would grow in higher percentages of milk solids at higher temperatures than at cooler temperatures (71). Ingredient effect is a total phenomenon. This is exemplified by the fact that egg protein will be more or less protective depending on the other ingredients present (19, 72). Pure proteins had little effect on salmonellae in one study. Peptides and amino acids provided only a minor amount of protection during heat treatment (64). Katamey and Khan found that fatty acids and triglycerides were bacteriocidal for salmonellae immediately with no additional changes after one week in the recovery medium (73). Pertinent research with egg proteins and milk solids verify the protective effect they may have in a heated medium (19, 71, 72, 74, 75). Evidence from several studies points to the fact that whole milk gave more protection for bacteria than all other substances tested, including various peptide mixtures, salts, or carbohydrates (24, 64).

NaCl, as a common food element, has an influence on the thermal resistance of bacteria. For example in one study, NaCl was responsible for thermal resistance six times greater than sugar for salmonellae in similar products. Glauert and Cotterill state this resistance was not explained by the viscosity of the solution, but possibly by cell dehydration (76). The effect of NaCl is in part determined by the temperature of a medium. Low levels of

NaCl (1 to 4 percent) stimulated the growth of salmonellae--the most stimulation occurring at temperatures lower than optimum for the microorganism (12 C.). Such low concentrations are probably not sufficient to prevent growth at higher temperatures (37 C.). NaCl concentrations of 7 to 8 percent caused growth increases at temperatures of 37 C. (77). High NaCl concentrations (up to 12 percent) decreased the production of staphylococci enterotoxins B and C to undetectable levels in a study by Genigeorgis and his coworkers (14, 78). The production of staphylococcal toxins was not influenced by the presence of NaCl in one study (8). Preservatives may have some effect on the thermal resistence of bacteria (79, 80), but this effect is not well understood.

A realization of the effect of ingredients on bacterial survival must necessarily include the potential bacterial status of raw materials put into food. Casseroles are particularly susceptible to many sources of organisms due to the array of constituents used. Sea foods are an excellent raw medium for bacterial growth (81). Raw vegetables, on the other hand, are seldom a problem because a cooking period long enough to soften the tissue is adequate to destroy surface bacteria. Acid-forming microorganisms on vegetable surfaces are competitive to salmonellae

(15). Meat products are of special importance in bacteriological quality because raw meat is a good growth medium for many harmful organisms. Studies with C. perfringens showed that 82 percent of veal cuts tested harbored this organism (48). Slanetz claimed that other raw meats contained C. perfringens as much as 17 percent of the time (82), and in 16 percent of all cuts examined in another study by Strong and her group (83). Staph. aureus was found in the highest incidence in pork chops (84). Salmonellae were isolated in high numbers from poultry, raw pork, and carcasses of other animals (12, 85, 86). The incidence of salmonellae on poultry products from the initial processing stages to the retail market stage showed a stable occurrence of the organism at all points. The percentages in finished products ranged from 12 percent to 58 percent (85, 87, 88, 89, 90, 91, 92, 93, 94, 95).

The role of the individual ingredients or the various foods in combination is not completely clear. That is, from the ingredients in a given food one cannot evaluate the total protective effect of a menstrum upon a microorganism. Dega et al. have suggested that S. typhimurium and other bacteria be tested in every food environment in order to understand the varied and total effects of a food substrate on an organism (71).

Competition of Microorganisms

Another factor which constitutes a viable safety mechanism in foods is that of naturally occurring competition of the food flora. This factor is one which has gained recent importance. Augmenting the aforementioned factors, natural bacterial competition affects the ability of a species to multiply. In a mixed bacterial population, organisms may stimulate or inhibit other organisms. In a discussion of bacterial competition, it should be noted that the competitors are considered to be the natural flora of the food. Food poisoning organisms are common flora on some foods, but are not considered desirable inhabitants of processed foods and could thus be considered to be foreign organisms to these foods. Results of studies indicate that competing bacteria (natural flora) most generally have an inhibitory rather than stimulative effect on foreign microorganisms. The full effect depends on all environmental conditions (96). Inhibition may be due to competition for amino acids or other nutrients. Some species such as B. cereus, E. coli, or Proteus vulgaris may produce antibiotic substances which inhibit other species such as Staph. aureus. Staph. aureus requires cystine, valine, glycine, proline, arginine, and aspartic acid. Serratia marcesans and Pseudomonas species inhibit Staph. aureus by outcompeting it for these nutrients. Competition for any of these amino acids will inhibit the species (97). The acid produced by the lactic acid microorganisms has value as an inhibitory substance for food poisoning strains which prefer a pH near neutrality (98). Indications are that the depletion of vital nutrients by competitors is a more important factor than the total proportion of Staph, aureus compared to competitors. Another major factor in the competition with Staph. aureus is the depletion of nicotanamide. Certain other organisms, if present, will metabolize this compound at a high rate, leaving insufficient amounts for Staph. aureus (99). Oxygen availability seems to be of little importance for competitive strains grew equally well in the center of the food mass as well as at the surface. There appears to be an indirect relationship between the concentration of effectors and the rapidity of retardation of foreign organism (100).

Peterson et al. stated that the possibility of staphylococcal food poisoning occurring after the consumption of a food containing mixed bacterial populations is very limited. The competitive inhibition will most likely occur between freezing temperatures and room temperatures (101). In the range of room temperature (about 25 C.) to 37 C. the saprophytic spoilage will render a product inedible in less than 24 hours. In this way it is unlikely that a viable staphylococcal population would be consumed (102). Very seldom was a staphylococcal population of 10^7 organisms per milliliter obtained when a food menstrum contained approximately equal numbers of repressors and inocula (100).

Ingredients play a role in bacterial competition also. This is an indirect asset to competitively destroying Staph. aureus. On the other hand, the role of ingredients in competition may be a favoring of growth of pathogens. This is demonstrated by NaCl concentrations which selectively inhibited competitors of Staph. aureus allowing profuse growth of the food poisoning organism (103). Saprophytes were retarded at all NaCl concentrations, while staphylococci survived well at high NaCl concentrations at 20 C., 30 C., and 37 C. The NaCl enhanced the staphylococcal population, while simultaneously repressing saprophytic growth. This decline in the competitor's ability to survive is contrary to the evidence mentioned earlier, again showing the variability of all factors in the ability of organisms to survive in foods.

Quality of the Finished Product

There are several places in the preparation of food where contamination is most likely to occur. The original raw products can add numerous bacteria of an undesirable nature. During the processing stage, food handlers and utensils offer a potential source of unwanted organisms. Proper cooking procedures should eliminate organisms from these two sources. The last major source is post-cooking contamination. In a discussion of processed foods, one must consider the quality of the raw products, the preparation stage, and the quality of the finished product. This section will concern itself with the bacteriological quality of the end product. There is not an abundance of research in this area.

One survey carried out by Jopke and Riley in the Minneapolis-St. Paul area involved itself with the quality of foods served from various cafeterias in the surrounding districts. Laboratory tests were done on such foods as creamed turkey, chow mein, noodle casseroles, and macaroni and cheese. No salmonellae or *C. perfringens* were found in any of the dishes tested. *Staph. aureus* was found in quantities of less than ten per gram which are numbers considered too small to be associated with disease outbreaks. Total counts varied from less than 300 per gram to 10^6 per gram (104). Imitation sandwich spreads were found to be poor bacteriological growth media in another study. Salad-type fillings for sandwiches had a much higher incidence of microorganisms than did cheese or ham fillings (105). A study of salads and sandwiches at retail outlets showed that 29 percent of the salads and 60 percent of the sandwiches contained coagulase-positive staphylococci. Salmonella and C. perfringens were found in much smaller numbers (106). A Kansas study deomonstrated an incidence of 3.5 percent coagulase-positive staphylococci in frozen cream pies (107).

Preparation as a Factor in Food Contamination

Microbial contamination can occur consistently during food preparation depending upon the practices employed. Good sanitation habits are an essential to any food establishment, and poor sanitary practices are a prime factor in food poisoning outbreaks. Consequently, good food handling procedures are a major preventative force in large quantity food establishments. A good sanitation program must include control of both primary and secondary sources of contamination. Primary control measures include the food handler himself, the food source, air, water, insects, and rodent contamination. Secondary sources are things such as equipment, utensils, and physical surroundings such as walls and other surfaces (2).

Man is a major source of microbial contamination. The average person in good health is a common reservoir of *Staph. aureus* and enterococci in the throat, mouth, and nose. A survey of fifty healthy food handlers showed forty harbored *Staph. aureus*. Of these staphylococcal isolates, 90 percent were from the nose, 34 percent from the hands, and 28 percent from both (108). Feces of 219 food handlers showed no salmonellae. However, 78 percent showed *C. perfringens* and 80 percent *E. coli* (75). Prepared foods had much higher counts than strictly fresh or raw foods, thus substantiating the implication of food handler contamination (109). The personal habits of food workers are very difficult to control. There are endless opportunities for a worker to contaminate his hands--either from touching his own body or from contact with other surfaces (110). Clothing can be a potential hazard in food sanitation because dangerous organisms can persist on fabrics under normal working conditions. *Staph. aureus* was tested on several textiles and was found to survive best at room temperature (25 C.) with a 35 percent relative humidity. Organisms did survive on cotton sheeting and other cottons (111). S. typhimurium survived the least time on wash and wear fabrics and cottons (112) at the same relative humidity (35 percent) and at room temperature.

The potential hazards of mistreated equipment cannot be disregarded. The danger lies in the careless misuse given them by handlers. Several days of outbreaks in a Naval station were traced later to a contaminated cutting board. Turkey had been thawed on the board so juices remained on it. The failure to clean it led to later food poisoning outbreaks because the cooked meat was sliced on the already contaminated board (113). Such items as dishcloths and other cleaning supplies can be the subtle source of harmful food organisms. Although there is a tendency to assume that disposable towels are sterile, this was found not to be so. Total counts on unused handwipes were less than 100 organisms per gram. Others contained as many as 7,206 organisms per gram. Of this flora, 90 percent were found to be Bacillus species (114). Some caution should be used when handling these handwipes around foods. Nelson did a study on restaurant counter cloths and found counts of more than one million organisms per four square inches. The organisms were mainly yeasts and molds, although some coliforms, staphylococci and enterococci were found (115).

Food preparation practices play a role in the presence or absence of harmful organisms in a prepared food. Some preparation methods can decrease the possibility of bacterial contamination more than others. For example, fewer salmonellae were found in poultry if it was deboned immediately after cooking instead of being cooled before deboning. The incidence was greater even if the food was held at 3 C. or -15 C. for as long as nine months and then deboned (116). Sandwiches made from filling, cooled several hours, showed less bacterial contamination than those from fillings spread immediately after being made (105).

The importance of complete food sanitation is clear. Good sanitation is the responsibility of both the worker and the food supervisors. Food service establishments have attempted sanitation programs with success. The supervisor must help the worker to appreciate his extreme importance in the health of the numerous people in the food chain. A good sanitation program should not limit the food handler. If well done these courses should create a cooperative attitude where rules become habits. Courses in food safety are potentially valuable, and will be successful only if well administered. Employees may reject any education if they are insulted or their role is negated, and thusly any cooperative effort is discouraged. One observer has formulated some pertinent questions concerning the appropriateness of food safety courses (117):

1. Is the time allotted adequate to cover the essential material?
2. Is the information recent?
3. Is the supervisor involved?
4. Is the information put to practical application?
5. Are the instructors good and do they show interest in the material they are presenting?

Etiology of Some Specific Outbreaks

The etiology of outbreaks in various institutions is important for stimulating awareness of foodborne illnesses and thusly preventing recurrences. Bacteria exhibit no discrimination--that is, they may strike at penal institutions, college cafeterias, cafes or hospitals. One hundred persons in a Michigan prison were afflicted by *S. enteriditis*. The cause was cross-contamination due to poor handwashing facilities, poor lighting, worn cutting boards, and inadequate heating of the foods involved (118). *S.*

newport was isolated in a New Mexico mental hospital after a Thanksgiving dinner in which inadequate time and temperature were used to roast turkey (119). Multiple contamination by S. thompson occurred in a church supper which affected 200 people. Chicken was thawed and handled improperly, and the potato salad and bread dressing were infected (120). Persons numbering 303 were left ill following the consumption of barbecued pork in a restaurant. S. thompson was present due to improper facilities and procedures, and poor sanitary practices which allowed survival, dissemination and replication of salmonellae (121). Soiled linen and poor hand washing procedures were responsible for five deaths and 43 illnesses in a Michigan nursing home. S. typhimurium was the prevalent isolate (122). These aforementioned outbreaks have all occurred within the last three years.

The importance of careful handling of food cannot be stressed enough. In recent years, 99 percent of all reported cases of staphylococcal and salmonellae outbreaks were due to cooked high protein foods. Leftovers were responsible for 94 percent of outbreaks (123). A salmonellae outbreak involving 390 persons in Kentucky was due to undercooked turkeys. These were later used to make creamed turkey (124). An outbreak at the University of Wisconsin was due to C. perfringens in the gravy. Day old beef stock was stored overnight in nine gallon containers which could not cool properly. This stock was then added to new gravy and boiling was inadequate to eliminate the danger (125). Adequately prepared foods are unlikely to harbor C. perfringens (83). Poor handling of turkeys both before and after cooking, allowed growth of S. typhimurium and C. perfringens. The same infected food was reused three times (126). Staph. aureus was isolated from a chicken salad from a school cafeteria serving 6,000 children. Intoxication occurred in 1,300 students because the salad was not properly chilled from the time the chickens were deboned until they were served (127).

Summary

The growth of harmful pathogens is affected by a rather complex set of environmental conditions. These factors are so interrelated that it is impossible to discuss one without the other. Temperature is probably the most important overall influence. It influences all other factors such as competing natural flora, ingredients and water activity. Raw materials are important for the natural flora they represent as well as their role as an ingredient factor with protein, carbohydrate or fat components. The total effect of these factors will be the determinant of whether microorganisms can grow. With so many factors having influence, it is impossible to make any all-inclusive rules concerning time-temperature for all foods.

Foods prepared in a food service establishment must progress through several stages. The cooking zone is essential to destroy pathogens and the holding zone should prevent multiplication though it does not usually destroy organisms. The chilling zone also should prevent increases in numbers over an extended period and probably counts will decrease. The danger zone is between the chilling and the cooking zone, and public health regulations designate that food should not be held in this zone any longer than possible. This zone is in the temperature range wherein foods are most susceptible to bacterial growth.

Proper sanitation practices are another essential which cannot be overlooked as a factor in determining food safety. Food handlers are the ultimate factors in food safety for they can choose quality ingredients which go into a food. They can also control other factors like temperatures used and cleanliness of equipment. They must be given proper consideration in any program of food sanitation.

The following study is intended to consider the food sanitary practices as a factor of food safety. A good measure of proper food safety is the bacteriological quality of the food itself following preparation. Casseroles were selected, as they represent a wide array of ingredients, handling procedures, and heating--several opportunities for the inclusion of pathogens. The dishes were evaluated microbiologically for incidence of *Staph. aureus*, *Salmonella*, and *C. perfringens*.

CHAPTER III

METHODS

This study is concerned with the qualitative microbiological evaluation of certain dormitory cafeteria hotdishes as an indicator of the sanitation practices in such a facility. The casseroles were selected for testing because their preparation encompasses a series of potentially hazardous steps in which contamination might occur. The making of a casserole involves a variety of raw goods, a substantial amount of human handling, and a heating process of some degree. Carelessness in one or more of these steps could result in a final product with harmful bacterial growth which is dangerous for human consumption. The casseroles selected for bacteriological examination were tuna noodle, beef stroganoff, and pork chop suey. Each dish was sampled from the cafeteria line, at the point where the food reaches the consumer. The purpose of the study was to evaluate the lengthened serving hours now observed in the cafeteria to see if the extended hours are of danger relative to maintenance of food safety. These augmented serving hours were instituted in the Montana State University food services in January of 1970. Foods are now served weekdays for eleven hours of the day compared to earlier totals of six to seven hours. There is an obvious lack of pertinent research in this particular area. This is partly due to the fact that this trend in food service management is relatively recent. At the present time, the lunch meal is served from 10 a.m. until 2 p.m. Mondays through Saturdays. The dinner meal begins at 4 p.m. and extends until 6:15 p.m. Mondays through Saturdays. Sundays are not included in this study. All the methods used for the experiment were acceptable methods and they will be reviewed in this section.

Preparation of Casseroles

Recipes of the casseroles studied are included in Appendix A. The methods all include a period of heating from one to one-and-one half hours in steam kettles in order to tenderize the ingredients, and distribute the flavors. At this point, the tuna noodle casserole was baked in the oven at 232 C. The other two dishes, once in serving pans, were not baked but were maintained in the thermal units directly behind the line until needed. The temperature of these electric units is 66 C. The steam tables are maintained at 82 C. in order that the food may be kept above 60 C.

Collection of Sample

Samples were gathered on each day that tuna noodle casserole, beef stroganoff, or pork chop suey appeared on the menu of one college cafeteria during the months of December, 1971, and January through April of 1972. The particular unit feeds approximately 1,760 students of both sexes. The particular dishes were offered at either lunch or dinner meals. During the meals when these casseroles were offered, a total of four samples were gathered. The initial sample preceeded the actual meal time. The remaining three samples came directly from plates served at the cafeteria line. Sample one was taken during the final preparation stage; that is, while the food was still in the steam kettles, heated, and ready to be put in serving pans. The first sample was taken from fifteen minutes to 1 1/2 hours before the meal period. Other samples followed at half hour to two hour intervals. Variations in the time of the initial sample were due to the type of food or situational delays in preparation. Beef stroganoff, for example, could not be sampled until the sour cream was added and the entire mixture was reheated. The sour cream was added as close to serving time as possible to prevent prolonged heating and thus separation of the cream from the mass.