ASSESSMENT OF BACTERIAL HAND CONTAMINATION AMONG FOOD HANDLERS WORKING IN THE STUDENT CAFETERIAS OF JIMMA UNIVERSITY MAIN CAMPUS, JIMMA, SOUTH WEST ETHIOPIA

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Abbreviations and Acronyms

ATCC: - American type culture controls

BPW: - Buffered Peptone Water

CDC: - Center for Disease Control and Prevention

FAO: – Food and Agricultural Organization

JU: -   Jimma University

KIA: - Krigler Iron Agar

LDC:- Lysine Decarboxylase

LIA:-   Lysine iron agar

MR: - Methyl Red

MSA: -Mannitol Salt Agar

NA: - Nutrient Agar

RV:- Rapport Vassiledes

SOP: - Standard operating procedure

Spp: - Species

TSI: - Triple Sugar Iron agar

UN: – United Nation.

VP: -Voges Proskaeur

WHO: – World Health Organization

XLD: - Xylose Lysine Deoxycholate agar

Abstract

Background: Food borne diseases continue to be a major global health problem and are the leading causes of morbidity and mortality in developing countries. Food handlers play a major role in the transmission of food borne pathogens via hands. This study aimed to assess the bacterial hand contamination among food handlers working in the student cafeterias of Jimma University main campus.

Methods and materials: A cross-sectional study was conducted from May 2012 to April 2013 among food handlers working in the student cafeterias of JU main campus. The data was collected by using structured questionnaire and observational checklist. Hand rinse samples was collected from participants and microbiologically examined for the presence of potential food borne bacterial contaminants using standard laboratory methods. The data was entered into a computer and analyzed using SPSS version-16.0 software.

Result: Among 230 food handlers, 114(49.6%) were tested positive for one or more potential food borne bacterial contaminants, and 73(31.7%) were tested positive for enteric pathogens. A total of 171 bacterial hand contaminants were isolated. *S. aureus* 54(23.5%), *Klebsiella spp.* 37(16.1%), *E.coli* 25 (10.9%), *Enterobacter spp.* 21(9.1%), *Citrobacter spp.* 10(4.3%), *Serratia marcescens* 6 (2.6%), *Pseudomonas aeruginosa* 8(3.5%), *Proteus spp.* 5(2.2%), *Providencia rettegri 3(1.3), and salmonella spp.* 2(0.9%) were isolated with their corresponding prevalence rate. Bacterial hand contamination rate have significant association with service years (Chi-square=13.732, DF=4, P=0.008), age ($\chi2$= 11.308, P=0.010) and cleanness of outer garments ($\chi2$=7.653, P=0.006).

Conclusion: The findings of this study emphasized the importance of food handlers' hands as a potential vector for potential food borne bacterial contaminants which could constitute a potential risk to food borne outbreaks. New employees and young and inexperienced food handlers should be well trained on personal hygienic practices pointing out on the importance of hand hygiene and appropriate hand washing techniques.

Keywords: Food handlers, Food borne bacterial contaminants, Isolation rate, Hand rinse

CHAPTER ONE: INTRODUCTION

1.1 Background

The availability of safe food improves the health of people and is a basic human right. Safe food contributes to health and productivity and provides an effective platform for development and poverty alleviation [1].Food acts nevertheless as a vehicle for the transmission of a variety of disease causing agents such as bacteria, parasites, viruses, toxins and chemical residues [2].

Foodborne diseases can be defined as diseases commonly transmitted through food. Foodborne diseases comprise a broad group of illnesses caused by microbial pathogens, parasites, chemical contaminants and biotoxins. The burden of disease can be defined as the incidence and prevalence of morbidity, disability, and mortality associated with acute and chronic manifestations of diseases [3].

The Centers for Disease Control and Prevention has identified more than 400 food-related illnesses. About two thirds of all outbreaks involve bacteria. The illnesses are caused either by the microorganisms themselves or by the toxins they release [4]. The consumption of foods contaminated by foodborne pathogenic microorganisms and toxins produced by them causes deaths, illnesses, hospitalization, and economic losses. Due to their widespread nature, foodborne diseases, in particular gastro-intestinal infections, represent a very large group of pathologies with a strong negative impact on public health [5].

Food borne diseases continue to be a major global health problem and are the leading causes of morbidity and mortality in both developed and developing countries. According to the 2011CDC estimates each year roughly 1 in 6 Americans (or 48 million people) gets sick, 128,000 are hospitalized, and 3,000 die of food borne diseases [6]. WHO estimated that in developed countries, up to 30% of the population suffers from food borne diseases each year. Moreover, in developing countries, up to an estimated 70% of cases of diarrheal diseases are associated with the consumption of contaminated food [7]. WHO/FAO estimate that approximately 2 million deaths are estimated per year from diarrheal diseases resulted mainly from poor sanitation and consumption of unsafe food [8].

The burden of all food-borne diseases is difficult to estimate but is likely to be significant. Food-borne diseases are a serious threat to people in Africa, causing an unbearable public health burden and massive economic losses. WHO estimates that some 700,000 deaths per year in Africa are due to food and water-borne diseases. These outbreaks only show the tip of the iceberg, as many more cases that are sporadic go unreported [2].

WHO and its Member States is recognizing food safety as a world-wide challenge. The true incidence of food-borne diseases is often difficult to evaluate. In many instances, only a small proportion of cases come to the notice of health authorities, and even fewer are investigated. It is believed that in industrialized countries less than 10% of the cases are reported, while in developing countries reported cases probably account for less than 1% of the total [9].

Many food-borne disease incidents are reported every year in Africa. Numerous factors, contribute to this high number of incidents. However, it is extremely important to note that most cases of food-borne disease in the region are not reported, so the true extent of the problem is unknown. In most countries of the region, the surveillance infrastructure for food-borne diseases of both microbiological and chemical etiology is weak or non-existent. This absence of reliable data on the burden of food-borne disease impedes understanding about its public health importance and prevents the development of risk based solutions to its management [2].

More aggravated situations and challenges prevail in Ethiopia where food safety issues are not well understood and have received little attention. In Ethiopia, according to the Ministry of Health annual report of 2011, dysentery and gastroenteritis were among the top ten diseases of outpatient visits although the report did not include all regions activity [10].

1.2. Statement of problem

Food-borne diseases represent a persistent global health burden, and food handlers play a major role in their transmission [11]. Even though the sources of food contamination are diverse, food handlers serve as important source of food contamination either as carriers of pathogens or through poor hygienic practices [12]. The mishandling of food and the disregard of hygienic measures enable pathogens to come into contact with food and, in some cases, to survive and multiply in sufficient numbers to cause illness in consumers. Personal hygiene and environmental sanitation are among the key factors in the transmission of food borne diseases (13).

Several food-borne disease outbreaks are associated with poor personal hygiene of people handling foodstuffs. CDC reported that approximately 20% of food-related infections are due to food handlers [14]. Another study conducted in Malaysia also showed that approximately 10-20% of food-borne disease outbreaks are due to contamination by the food handlers [13]. Food workers may transmit pathogens to food coming from a contaminated surface of another food, or from hands contaminated with organisms from their gastrointestinal tract. This is also supported by report in which about 89% of outbreaks caused by food contamination by food workers, pathogens were transferred to food by workers' hands by [15].

Food handlers are the most important sources for the transfer of microbial pathogens to food either from their hair, skin, hand, digestive systems, respiratory tracts, or from contaminated food prepared and served by them [16, 17]. The hands are the last line of defence against exposure to pathogens which can occur either directly from the hand to the mouth, eye, nose, or other area of the skin, or indirectly by "handling" of food or water. The hands are particularly important since they are the last line of defence in the chain of transmission of gastrointestinal pathogens, either directly from hand-to-mouth, or indirectly by "handling" of food or water [18].

The hands of food handlers can be the vector to spread harmful microorganism through cross contamination, and during or after they experience gastrointestinal infection. An employee might contaminate his hands when using toilet, or bacteria might be spread from raw foods, from contaminated equipments, and environment [19]. Thus, these contaminated hands can transfer intestinal microbes to foods, equipment, and other workers in the food storage and preparation areas unless correct personal hygiene and adequate hand washing procedures are followed [21,

22]. Some of the bacteria that can colonize the hands of food handlers are Escherichia coli and Staphylococcus aureus [20].

WHO emphasizes that "outbreaks of food-borne diseases can be reduced if both professional and domestic food-handlers understand the importance of correct hygienic food practices [12]. Food handlers should not smoke, sneeze, spit, cough, eat, handle money or engage in any act that could contaminate the food during the performance of their activities [23]. The role of the hands in disease transmission and the importance of hand hygiene in controlling infection in the food establishment are well established. Hand washing has been identified as the single most important means of preventing the spread of infection and if poorly or improperly implemented, can lead to foodborne illness outbreaks [23, 24].

Certain characteristics concerning these professionals, such as poor educational level, sex, low socioeconomic level, rapid staff turnover, literacy as well as poor motivation due to low pay, experience, training and job status, can contribute to poor professional performance at work [19].

Food handlers in bigger eating establishments cater to a larger number of people, they are epidemiologically more important than domestic food handlers in spreading of food borne disease [11]. Nonetheless, bacterial hand contamination of food-handlers, may pose a real threat to those who are more susceptible to infection. Studying the hands microbial flora among the food handlers could have paramount importance to understand the hygienic practices of food handlers. The presence or absence of bacteria in the hands of food handlers can be used as a quantitative indicator of their behavior regarding food-related and personal hygiene [25]. There are few related studies in Ethiopia and specifically to this study area. Therefore, this study aimed at assessing the bacterial hand contamination among food handlers working in the student cafeterias of JU main campus.

CHAPTER TWO

2.1 Literature review

According to the 2011CDC estimates each year roughly 1 in 6 Americans (or 48 million people) gets sick, 128,000 are hospitalized, and 3,000 die of food borne diseases. Eight known pathogens account for the vast majority of illnesses, hospitalizations, and deaths, of which six are bacteria: *Salmonella, non typhoidal, Staphylococcus aureus, E. coli (STEC) O157, Campylobacter species, Listeria monocytogenes, & Clostridium perfringens* [6].

Food handlers may transmit pathogens passively from contaminated sources to food. They may be sources of organisms either during the course of gastrointestinal illness or during and after convalescence, when they no longer have symptoms. During the acute stages of gastroenteritis large numbers of organisms is excreted and by the nature of the disease are likely to be widely dispersed; clearly, food handlers who are symptomatically ill may present a real hazard and should be excluded from work. Good hygiene, both personal and in food handling practices, is the basis for preventing the transmission of pathogens from food handling personnel to consumer [19].

The hands of food service employees can be vectors in the spread of food borne diseases because of poor personal hygiene or cross-contamination. For example, an employee might contaminate his hands when using the toilet, or bacteria might be spread from raw meat to salad greens by food handler's hands, point out that data on risk factors for food borne diseases imply that most outbreaks result from improper food handling practices [19].

Food Handlers should always wash their hands when their level of cleanliness may affect food quality; for example: just before food handling, after any interruption, after touching contaminated material, after using the bathroom and whenever else needed. Food handlers should tie should cover hair and wear appropriate protective covering , cut their fingernails short and during handling they should remove jewelry from their hands. They should not smoke, sneeze, spit, cough, eat, handle money or engage in any act that could contaminate the food during the performance of their activities [23].

When food handlers do not practice proper personal hygiene or correct food preparation, they may become vehicles for microorganisms, through their hands, cuts or sores, mouth, skin and hair, among others. It is known that improper handling is one of the main causes of food borne disease and that inappropriate hand hygiene is an important risk factor for food contamination [27]. The mishandling of food and the disregard of hygienic measures enable pathogens to come into contact with food and, in some cases, to survive and multiply in sufficient numbers to cause illness in consumers. Personal hygiene and environmental sanitation are key factors in the transmission of food borne diseases [13].

A cross-sectional study done in Ambo town showed, among interviewed food handlers only forty (28.6%) of food handlers had worn appropriate outer garment and hair covers, on the other hand sixty-seven (47.9%) of food handlers were not kept their personal hygiene and cleanliness of their overcoat. It was observed that 38(27.1%) of food handlers wore rings/finger ornaments on their finger during food preparations. 42(30%) of food handlers fingernail not short trimmed and clean. 8(5.7%) of food handlers responded that they were not washed their hands before starting of food handling [28].

Persons working in food services have to go through medical examination every three months and obtain a report indicating that they may work in such services [29]. A study in Bahardar shows, almost all (90.6%) food handlers had a habit of hand washing after toilet. However, a few number (11.2%) of food handlers had a habit of hand washing after touching dirty materials and different body parts (hair, nose and ear) between handling of food items. None of the participants had medical checkup including stool examination. Fifty four (14%) food handlers were certified for training in food handling and preparation [30]. Food handlers should receive training before starting work in any establishment, with a periodic refreshing training there after [31].

According to study done at Mekele University among the total respondents, 195 (70.4%) of them had a habit of hand washing with soap or plain water particularly after toilet. Almost half of the respondents, 51.5% wash their hands after cleaning blowing nose, coughing and sneezing, and 202(72.9%) used soap to wash their hands before preparing food. The majority, 245(88.4%) reported that they kept their fingernail cut short. Beside this, only 34 (12.3%) respondents were certified with six months formal food handler's training program from different training centers. Whereas, 63.2% of food handlers have got medical checkups [32].

According to a cross-sectional study conducted on Mekele town catering establishments, two hundred one (72.6%) of food handlers were found wearing outer working garments, of which 67(33.3%) of the food handlers outer garment were not clean. One hundred and eight (39%) were found with covered hair, and 211(76.2%) were with trimmed fingernails and 99(35.7%) were found preparing food while they worn finger ornaments. Only 63 (22.7%) food handlers took medical check up in the past one year. Diarrhea, respiratory infection, skin lesion and nose and eye discharges were observed on 25 (9%) of the food handlers [33].

The microbiological flora of the skin can be divided into residents and transients; the residents are the normal population and are generally not a health hazard. The transients, which may include pathogenic organisms, are picked up onto the skin surface and transferred around the environment [34]. The only pathogenic microorganism in the permanent group of bacteria associated with the human skin is *S. aureus*. It is not possible to fix an acceptable contamination level for *S. aureus* after proper hand washing [35, 36].

Resident skin bacteria survive longer on intact skin than do gram-negative transient species. The protective function of the resident flora is colonization resistance, through microbial antagonism and competition for nutrients in the ecosystem. The dominant species is Staphylococcus epidermides, which is found on almost every hand. Other regular residents are *Staphylococcus hominis* and other coagulase-negative staphylococci, followed by *propionibacteria, corynebacteria, and micrococci*. The transient skin flora consists of bacteria, fungi, and viruses that may be found on the skin only at times. The transmissibility of transient bacteria depends on the species, the number of bacteria on the hand, their survival on skin, and the dermal water content [37].

The transient microorganisms found on hands vary significantly according to the surfaces contacted, and that there are microorganisms characteristic for skin, respiratory system, stool, and peri-anal region [35]. The transmissibility of these bacteria depends on the species, the number of bacteria on the hand, their survival on skin, and the dermal water content [37].

There is a range of sampling techniques, including contact plates and rinse methods, and different results may be obtained depending on the method used. The contact methods measure

the aggregates or micro-colonies of cells while scrub rinse methods measure the total viable cell population resulting from the dispersal of colonies [34].

Food workers may transmit pathogens to food coming from a contaminated surface of another food, or from hands contaminated with organisms from their gastrointestinal tract. The Enterobacteriacea is constituted by gram-negative bacilli which can inhabit human and other animal's intestines. Enteric pathogens that are believed to be capable of being transmitted by food workers include, but are not limited to, *E. coli*, *Salmonella spp., Shigella spp*. *E. coli* is transmitted via the faecal–oral route and is used as indicator of recent faecal contamination. The organism is naturally found in the human intestine and although most strains are harmless some, such as serotype 0157:H7 can cause serious illness [46].

In addition, pathogens such as *Yersinia, Proteus,* and *Klebsiella*, which could originate from raw animal products, can contaminate hands and from where it can be transferred to foods, equipment and other workers. These bacteria represent 70–80% of gram-negative microorganisms isolated from infectious diseases. Some of them are also recognized as indicators of fecal contamination in foods [15].

The food handlers that are both reservoirs and vectors of microorganisms can act as a source of microbial contamination of food products. The hands are the major source of infection from microorganisms [19]. According to Study done on teaching hospital food handlers, the most common species were Coagulase (-) *Staphylococci* 74 (95%), *Staphylococcus aureus* 58 (74%), *Enterococci* 15 (21%), *Diphteroid bacilli* 15 (19%) and *E. coli* 3 (3.9%) were found on the hands [25].

Enteric pathogens are among the transient hand flora that can be easily removed by hand washing. Isolation of these organisms includes a faeces-to-hand spread and indicating a poor hygiene is practiced by the food handlers [36, 38]. Their presence indicates fecal contamination and food handlers are not taking enough care in hand hygiene [39]. Microbiological analyses of the food handlers' hands in municipal public schools of Natal, Brazil, by administrative district, showed 55.6% (45/81) fecal Colliform contamination on the hands [27]. In a similar study on microbiological risk factors associated with food handlers in elementary schools from Brazil, *Enterobacter spp.* (54.5%), *Serratia spp.* (9.0%), *Shigella spp.* (9.0%), *E. coli* (6.8%),

Salmonella spp. (2.3%) and *Yersinia spp.* (2.3%), *Pseudomonas aeruginosa* (2.3%) were isolated from the hands of food handlers [40].

According to study on microbial analyses of the Low-income Puerto Rican Meal Preparers hands demonstrated that 91% tested positive for any type of bacteria and 38% were positive for Coliforms. *S. aureus* was found on the hands of 42% of participants. However, none of the participants' hands were found to be positive for *Campylobacter, Listeria*, or *Salmonella* genera [41].

Study on Iranian food handlers showed that, the hands of 109 people out of 150 food handlers (72.7%) were contaminated with one or more potentially pathogenic bacteria. The results also showed that 64.1% (111 out of 173 total cases) of microorganisms isolated from the hands of food workers were Gram negative bacilli. The most common potentially pathogenic bacteria isolated from hands of Iranian food handlers were *Bacillus spp.* (28.6%), *Escherichia coli* (22%), *Entrobacter spp.* (14.6%), *Klebsiella spp.* (13.3%) and *S. aureus* (12.6%) [36].

A cross-sectional study conducted in a rural area of Wardha district of Central India, amongst food handlers *Staphylococcus* in 91(56.87%), *E. coli* in 28(17.5%), and *Klebsiella* in 35(21.87 %) have been isolated of the nail cultures [42].

Study done on hands of hospital food handlers a total of 16 different bacteria were isolated, of which the most common was *S. aureus* 70%, followed by coagulase-negative staphylococci 56.7%, *diphtheroid bacilli* 21.7%, Bacillus spp. 10.5%, and *Escherichia coli* 7.8%. In this study, 93.3% of the hand samples were determined to be containing bacteria, with each harboring 1–5 bacterial species. In this study, Two hundred bacteria were isolated from hands of the inexperienced food handlers whereas 169 bacteria were isolated from experienced ones (t¼ 2:024; p < 0:05). These results indicated that there is a positive correlation between work experience and hand hygiene. Hand hygiene levels of the food handlers who had more work experience (>10 years) were better than inexperienced ones [39].

A study on assessment of hand washing facilities, personal hygiene and the bacteriological quality of hand washes in some grocery and dairy shops in Alexandria, Egypt. The bacteriological profile of the handlers 'hand washes was found contaminated with S.aureus in 31.0%, and fecal coliforms in 6.9% of the hand wash samples. [43].

According to study on prevalence of intestinal parasites and bacteria among food handlers in a tertiary care hospital, Mekkah, Saudi Arabia. Bacterial species isolated from cultures of fingernail contents of 200 samples were found to be coagulase-negative *staphylococci* 79 (39.5%) followed by *Staphylococcus aureus* 35 (17.5%), *Klebssiella species* 14(7%), E.coli 5 (2.5%) , *Serratia species* 4(2%), *Citrobacter species* 3 (1.5%), *Enterobacter species* 1(0.5%). However, no intestinal parasites were detected from the samples of fingernail contents [44]. In a similar study among food handlers in the Federal Capital Territory of Nigeria, the frequency and type of bacteria isolated from fingernail content of the 168 food-handlers studied. Bacteria isolated include *E. coli* (1.8%), *coagulase* negative *staphylococcus* (17.9%), *Staphylococcus aureus (7.1%), Klebsiella species* (2.4%), *Serratia species (1.2%), Citrobacter species* (1.2%), *Enterococcus species* (1.8%), while no bacteria was isolated from the finger nail content of 66.7% of participants. Also no parasite was isolated from majority of participants (98.2%), only 1.8% had *A. lumbricoides* isolated from their fingernail content [45].

E. coli is transmitted via the faecal–oral route and is used as indicator of recent faecal contamination. The organism is naturally found in the human intestine and although most strains are harmless some, such as serotype 0157:H7 can cause serious illness [46]. *E.coli* is normally absent from hands and the presence of *E.coli* is thought to give a better indication of fecal contamination enteric pathogens. A Study from South Africa reported the presence of Coliforms on 40% of food handler's hands, and enterobacteriaceae were present on the hands of food handlers (44%). *S. aureus* were present on 88% of hands [20].

Staphylococci are ubiquitously distributed in the environment and strains present in the nose often contaminate the back of hands, fingers and face, and nasal carriers could therefore easily become skin carriers. Information from staphylococcal food poisoning outbreaks indicates that strains of *Staphylococcus aureus* isolated from specimens of vomitus and faeces were identical with those from the implicated food and from the hands and often the nose of a food handler [42].Toxin-producing strains of *S. aureus* are the leading cause of gastroenteritis following handling of food by persons who carry the microorganism in their noses and skin. Study on prevalence and Significance of *S. aureus* and Enterobacteriaceae species in Selected Dairy Products and Handlers revealed out that the prevalence rates of *S. aureus* in hand and nasal swabs collected from dairy handlers were 60 and 70%, respectively [48].

In study carried out in Ethiopia, determined the prevalence of bacteria and intestinal parasites in fingernails contents among 127 food-handlers working in the cafeterias of a university and teachers training college, in Gondar. Hand washing habit after toilet was practiced y 89% of food handlers. In this study variety of bacteria were isolated from the fingernail contents of the food handlers. The predominant species isolated from fingernails contents were coagulase-negative *staphylococci* (41.7%), followed by *Staphylococcus aureus* (16.5%), *Klebsiella species* (5.5%), *E. coli* (3.1%), *Enterobacter species* (0.8%), *Serratia species* (1.58%), *Citrobacter species* (0.8%), and *Enterobacter species* (0.8%) from their fingernail contents [49].

In one study, multivariate analysis suggested that wearing rings was a major risk factor for carrying Gram-negative bacilli and *S. aureus* on hands, both being important nosocomial pathogens. There is also evidence that the organisms found under rings may be carried for many months [50]. Most infection control guidelines recommend that fingernails should be kept short. This facilitates cleaning but it has also been shown that longer nails have increased numbers of microorganisms. The subungual region contains large numbers of bacteria which are largely inaccessible during hand hygiene practices and are therefore difficult to clean compared with the rest of the hands [38].

2.2 Significance of the study

Food handlers play a major role in the transmission of food borne pathogens. The hands are particularly important since they are the last line of defence in the chain of transmission of gastrointestinal pathogens, either directly from hand-to-mouth, or indirectly by "handling" of food or water. Studying the hands microbial flora among the food handlers could have paramount importance to understand the hygienic practices of food handlers. The presence or absence of bacteria in the hands of food handlers can be used as a quantitative indicator of their behavior regarding food-related and personal hygiene. However this issue is not well studied in Ethiopia. So this study aimed to assess the bacterial hand contamination among food handlers working in the student cafeterias of JU main campus. Thus findings of this study could create awareness about food handlers' bacterial hand contamination status. The findings of this study may also help the responsible bodies to create and implement intervention programs. It will have an important implication for future development of hygiene legislations. Furthermore it can also be used as a reference and spring bond for further studies and planning programmers.

CHAPTER THREE: OBJECTIVES

3.1 General Objective

To assess the bacterial hand contamination among food handlers working in the student cafeterias of Jimma University main campus

3.2 Specific objectives

To determine bacterial hand ccontamination rate

To determine the types and prevalence of potential food borne bacterial hand contaminants

To assess factors associated with bacterial hand contamination

CHAPTER FOUR: METHODS AND MATERIALS

4.1 Study area and period

The study was conducted at student cafeterias in JU main campus, Jimma town, located at 355km southwest Ethiopia from May 2012 to April 2013. Its geographical coordinates are: $07^0 39$' Latitude and $36^0 50$' Longitude, at an altitude of 1700-1750m above sea level. Jimma is generally characterized by warm climate with an annual temperature range of $11.5^0 C$ and a mean annual rainfall of 1749 mm. Jimma University is organized into six colleges, out of which four of them are located in the JU main campus. Jimma University enrolls over 32,000 students in three campuses. The majority of students of the university are in Main campus. Around 500 food handlers are currently working in the student cafeterias of JU main campus.

4.2. Study design

Descriptive cross-sectional study design was used

4.3. Population.

4.3.1. Source population

All food handlers working in the student cafeterias of Jimma University main campus

4.3.2. Study population:

Selected food handlers working in the student cafeterias of Jimma University main campus

4.4 Eligibility criteria

4.4.1 Inclusion criteria:

 Food handlers who are engaged in food preparation, serving, and Cleaning

4.4.2. Exclusion criteria

Food handlers who have skin irritation, eczema, and inflammation

4.5 Sample size determination and sampling techniques

4.5.1 Sample size determination

Sample size (n) was determined using a formula to estimate single population proportion. Taking 50% prevalence of bacterial hand contamination (p=0.5), 95% confidence interval (z= 1.96) and 5% marginal error (d=0.05) the initial sample size was:

$$n = \frac{(z_{\alpha/2})^2 * P(1-P)}{d^2} = \frac{(1.96)^2 * (0.5)^2}{(0.05)^2} \approx 384 \text{ , where:}$$

P- bacterial hand contamination rate

z − z-statistics value that corresponds to 97.5 percentile cut-off point in the standard normal distribution

d − Margin of error

Since the total number of the source population was 500, correction formula was used to adjust the sample size to the population size and the sample size was calculated further as

$$\frac{n}{1 + \frac{n}{N}} = \frac{384}{1 + \frac{384}{500}} \approx 218$$

Finally by considering a 10% ($\approx$22 subjects) non response rate, the final sample size was determined as:

$$n + 22 \approx 240$$

4.5.2 Sampling technique:

Simple random sampling technique was employed. Study participants were selected by lottery method from the roster lists of food handlers which was obtained from students' cafeteria offices of Jimma University main campus.

4.6 Variables of the study

4.6.1 Dependent variables

Bacterial hand contamination

Types of bacterial hand contaminants

4.6.2 Independent variables

Sex

Age

Service years

Educational background

Salary

Hand washing habit

Fingernail status

Presence of jewelers on fingers

Regular medical checkup

Hygiene training

Outer protective coat, and Hair cover

Cleanness of outer garments

4.7 Data collection and laboratory processing

4.7.1 Data and specimen collection

Data related to socio-demographic characteristics, and personal hygiene practices of food handlers was collected by face to face interview method using structured questionnaire, and observational checklist. The tool was first prepared in English and then translated into the national language. Three sanitarians and two medical laboratory professionals were recruited for data collection and microbiological analysis. The data collectors were trained for two days by the principal investigator on data, and specimen collection procedures. After interviewing participants were asked to give hand rinse samples in sterile plastic bag (Stomacher@400 Classic; Seward, Worthing, UK) for microbiological analysis.

Before starting any meal preparation activity including hand washing (if any), participant's hands were sampled for microbial testing. Notification was not given in advance, and extra hand hygiene was not allowed during the hand rinse sample collection. A sterile polyethylene plastic bag technique was employed to collect the hand rinse samples. Participants were asked to dip their hands into sterile polyethylene plastic bag containing 100 milliliter of buffered peptone water (0.1% BPW) (Difco/Becton Dickinson, Franklin Lakes, NJ). The bag was grasped tightly around the participant's wrist and the Peptone buffer was massaged through the wall of the bag by the investigator for one 1 minute, over all surfaces of the participant's hand, particularly around the nails and palm. The bag was immediately sealed and transported to Jimma University medical microbiology laboratory for examination [50, 51].

4.7.2 Isolation and identification of potential food borne bacterial pathogens

All media used in this study were from Oxoid Ltd. and were prepared according to the manufacturer's instructions. The hand rinse sample were vortexed for one minute prior to microbiological examination. A loop full of each sample was separately spread-plated onto MacConkey (for detection of enteric bacteria), MSA (for detection of *staphylococci*), and XLD (for detection of *Salmonella*, and *Shigella*). Rappaport vassiliadis (RV) was used as a primary enrichment for the identification of Salmonella and Shigella. The bacterial colonies grown on the agar media were presumptively identified by colonial morphology and gram staining and a battery of biochemical tests like reaction on oxidase, catalase, simmon citrate, indole production,

urease, motility, coagulase, methyl red-voges proskaeur (MR-VP), LDC, KIA, gas and Hydrogen Sulfide (H$_2$S) production [52, 53].

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                        ┌──────────────────────┐
                        │ Hand  rinse sample,  │
                        │       100 ml         │
                        └──────────────────────┘

┌────────────────────┐  ┌────────────────────┐  ┌────────────────────┐
│ Inoculate  a loopfull│  │ inooculate a loopfull│  │ Enrich in 10 ml RV │
│ onto macconkey agar │  │     onto MSA        │  │                    │
└────────────────────┘  └────────────────────┘  └────────────────────┘

┌────────────────────┐  ┌────────────────────┐  ┌────────────────────┐
│ Indole, MR-VP,      │  │   Sub-culture to    │  │        XLD         │
│ Urease, SIM,        │  │   Nutrient Agar     │  │                    │
│ Kia, LDC, Oxidase   │  │                     │  │                    │
└────────────────────┘  └────────────────────┘  └────────────────────┘

   ┌────────────────────┐  ┌────────────────────┐  ┌────────────────────┐
   │ Enterobacteriaceae, │  │   Catalase and      │  │ Urease, KIA, SIM, LDC│
   │ and P. aeroginosa   │  │  Coagulase tests    │  │                    │
   └────────────────────┘  └────────────────────┘  └────────────────────┘

               ┌────────────────┐  ┌──────────────────────┐
               │   S.aureaus    │  │  Salmonella, Shigella │
               └────────────────┘  └──────────────────────┘
```
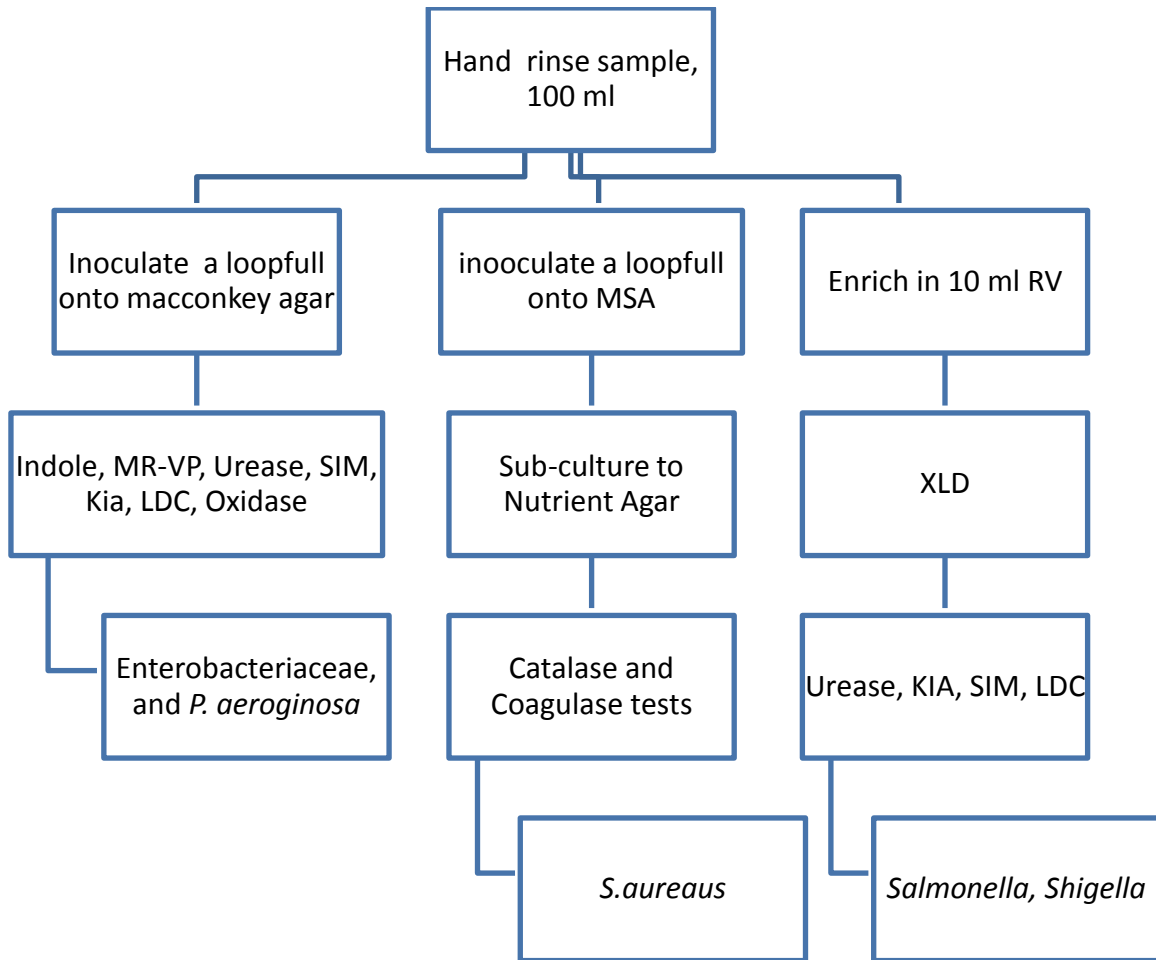
Figure-1: Simplified laboratory flow chart for identification of potential food borne bacterial hand contaminants

4.8 Quality control

To manage the quality of the work SOP was strictly followed during processing of each sample. All the instruments used for sample processing were checked for proper functioning as far quality control strains of *S. aureus* (ATCC 6538) and *E. coli* (ATCC 25922) were used. Data consistency and completeness were made all the way during data collection, data entry and analysis. Culture Medias were prepared based on the manufactures instruction. Then the sterility of culture media was checked by incubating 5% of the batch at $35 - 37^{o}c$ overnight and observed for bacterial growth. Those Media which shows growth was discarded.

4.9 Data processing and Analysis

All components of data were entered and cleaned, coded and analyzed using SPSS version 16.0 (Copyright (c) SPSS Inc., 1997-2007, Polar Engineering and Consulting.) computer software. Data was organized, summarized, and presented in simple descriptive Statistical methods. Chi-square test was used for checking any possible association between various categorical variables, and p-value <0.05 considered as significant. The findings of the study were compared to other studies.

4.10 Ethical consideration

The study was conducted after obtaining ethical clearance from Jimma University College of public health and Medical Sciences Ethical review Board. Permission letter was obtained from Jimma University Students' Service Dean Office to students' cafeteria office. Informed consent was obtained from participants after explanation of the purpose of the study and procedure of sample collection. In addition, participation in the study was made by willingness of study participants. All personal information about the study participants were kept confidential.

4.11 Dissemination of the findings

The finding of the study was disseminated to college of medical sciences and public health, Jimma University as a requirement for partial fulfillment of graduate study. The copy of the thesis results was provided to Student Cafeteria Offices of JU main campus. Furthermore a copy of the study will be submitted to health science library and Department of Medical Laboratory Sciences and Pathology. Finally, study results will be sent to the respective scientific journals requesting for publication.

4.12 Operational definitions

Bacterial hand contamination: presence of one or more potential food borne bacterial hand contaminants

Food borne diseases - intoxication, infection, or illness contracted by the consumption of contaminated food

Food handler - a person who is engaged in the process of food preparing, serving, cleaning, and etc.

Potential food borne bacterial contaminants – bacterial pathogens that can cause food contamination or spoilage

Personal hygiene - refers to those protection measures primarily with the responsibility of the individual, which promote and limit the spread of infectious disease, like hand washing using soap and water, keep body clean etc

Risk factor: - a factor whose presence is associated with an increased probability of bacterial hand contamination

CHAPTER FIVE: RESULT

5.1. Socio demographic characteristics of participants

Two hundred thirty food handlers were participated in this study making a response rate of 95.83%. From these 194(84.3%) of participants were females while 36(7.7%) were males. The mean and median age of the study subjects were 28.65(SD=8.09), and 26 respectively, where as the minimum and maximum ages were 18 and 55 years respectively. About half of the food handlers 119(51.7%) were single, while 86(37%) of were married. The educational background is found as no formal education 28(12.2%), elementary school 115(50%), secondary school 72(31.3%), high school and above 15(6.5%). Regarding their job position 73(31.7%) were Cookers, 99(41.3%) were servers and 58(25.2%) were cleaners. Concerning service years, majority 185 (63%) of food handlers have served for a period of less than five years in the student cafeterias of JU.

Table-1: Distribution of socio-demographic characteristics of food handlers working in the student cafeterias of JU main campus, May 2012-Aprill 2013

Variables	Categories	Frequency	Percent (%)
Sex	Male	36	15.7
	Female	194	84.3
Age	≤20 years	33	14.4
	21-30 years	124	53.9
	31-40 years	47	20.4
	≥41	26	11.3
Marital-status	Single	119	51.7
	Married	86	37.4
	Divorced	25	10.9
Ethnicity	Oromo	89	38.7
	Amhara	56	24.3
	Gurage	18	7.8
	Dawuro	32	13.9
	Kafa	16	7.0
	Others	19	8.3*
Religion	Orthodox	118	51.3
	Muslim	60	26.1
	Protestant	52	22.6
Education	No formal education	28	12.2
	Elementary	115	50.0
	Secondary	72	31.3
	High-school and above	15	6.5
Job-position	Cook	73	31.7
	Waiter	99	43.1
	Cleaner	58	25.2
Service years	<2	58	25.2
	2-4	87	37.8
	5-7	40	17.4
	8-10	25	10.9
	>10	19	8.7
Salary	420	160	69.6
	470	70	30.4

Key: (*) = implies to Wolayta, Sidama, Tigre and Yem

5.2. Personal hygiene practices of food handlers

In the present study out of the total participants 164 (71.3%) have worn outer protective Coat, 93(40.4%) have worn appropriate hair cover, 120 (52.2%) have kept their outer garments clean. Out of the total participants 160 (69.6%) have got informal food hygiene training, 131(57%) have got regular medical checkups, 185(80.4%) have trimmed fingernails, and 58(25.2%) have worn rings. Out of the total participants hand washing habit using soap and water is reported by 177 (77%) after toilet, 132 (57.4%) after touching dirty materials, and 201(57%) before food handling.

Table-2: Personal hygiene practices of food handlers working in the student cafeterias of JU main campus, May 2012 to April 2013.

Variables	Categories	Frequency	Percent (%)
Appropriate outer protective Coat	Observed	164	71.3
	Not observed	66	28.7
Hair cover	Observed	93	40.4
	Not observed	137	59.6
Cleanness of outer garments	Kept	120	52.2
	Not kept	110	47.8
Hand washing habit after toilet	With soap and water	177	77.0
	With water only	53	23.0
Hand washing habit after touching dirty materials	With soap and water	132	57.4
	With water only	98	42.6
Hand washing habit before handling food	With soap and water	201	87.4
	With water only	29	12.6
Finger nail status	Trimmed	185	80.4
	Semi-trimmed	45	19.6
Regular Medical Checkup	Checked	131	57.0
	Not checked	99	43.0
Presence of rings	Observed	58	25.2
	Not observed	172	74.8
Hygiene training	Trained	160	69.6
	Not trained	70	30.4

5.3. Types and prevalence of potential food borne bacterial hand contaminants

Bacteriological investigation of 230 hand rinse samples was performed out of which 114(49.6%) tested positive for one or more potential food borne bacterial hand contaminants, and 73(31.7%) were tested positive for Enteric pathogens. A total of 171 bacteria were isolated from which 109 (63.7%) cases belong to the family of Enterobacteriaceae. In this study the following bacteria's were isolated with the corresponding prevalence rate: *Staphylococcus aureus* 54(23.5%), *Klebsiella spp.* 37(16.1%), *E. coli* 25(10.9%), and *Enterobacter spp.* 21(9.1%), *Citrobacter spp.* 10(4.3%), *Pseudomonas aeruginosa* 8(3.5%), *Serratia marcescens* 6(2.6%), *Proteus spp.* 5(2.2%), *Providencia rettegri* 3(1.3%), and *salmonella species* 2(0.9%). While no *shigella species* was isolated.

Table-3: Type and prevalence of potential food borne bacterial hand contaminants among food-handlers working in the student cafeterias of JU main campus, May 2012 to April 2013

Bacterial isolates	Frequency (n=171)	Percent (%) (N=230)
S. aureus	54	23.5
E.coli	25	10.9
Klebsiella spp.	37	16.1
Enterobacter spp.	21	9.1
Citrobacter spp.	10	4.3
Proteus spp.	5	2.2
Serratia marcescens	6	2.6
Providencia rettegri	3	1.3
Pseudomonas aeruginosa	8	3.5
Salmonella spp.	2	0.9

5.4. Factors associated with bacterial hand contamination

In the present study, no statistical association was found between bacterial hand contamination rate, and gender, educational background, job position, medical check-up, food hygiene training, hand washing habit, and fingernail status. However, bacterial hand contamination rate have significant association with service years, age, and cleanness of outer garments.

The isolation rate of potential food borne bacterial hand contaminants was much higher 25(75.8%) among food-handlers of ≤20 years age group, and lower 10(38.5%) among those ≥ 41 years age group. There is significant difference in isolation rate of potential food borne bacterial hand contaminants by age groups (Chi-square=11.308, DF=3, P =0.01).

Bacterial hand contamination rate have significant association with service years of participants ($\chi 2$=13.732, DF=4, P=0.008). The isolation rate of potential food borne bacterial hand contaminants was relatively higher 39(67.2%) among food handlers served for a period of less than two years, and lower 6(30%) among those served for a period of greater than 10 years.

The isolation rate of bacterial hand contaminants was lower 49 (40.8%) among participants with clean outer garments, compared to 65 (59.1%) with unclean outer garments. There is significant association between bacterial hand contamination rate and cleanness of outer garments ($\chi 2$=7.653, P=0.006).

Table-4: Bacterial hand contamination rate and associated factors among food handlers working in the student cafeterias of JU main campus, May 2012 to April 2013.

Variables	Categories	Hand rinse culture result			P-value
		Positive N (%)	Negative N (%)	Total N (%)	
Gender	Female	98(50.5)	96 (49.5)	194(84.3)	χ2=0.448,
	Male	16(44.4)	20 (55.6)	36 (15.7)	P = 0.503
Age Group	≤20	25(75.8)	8(24.2)	33 (14.4)	χ2= 11.308,
	21-30	56(45.2)	68(54.8)	124 (53.9)	P=0.010
	31-40	23(48.9)	24(51.1)	47 (20.4)	
	≥41	10(38.5)	16(61.5)	26 (11.3)	
Educational background	No formal education	14(50.0)	14 (50.0)	28 (12.2.)	χ2 =2.176,
	Elementary school	62 (53.9)	53 (46.1)	115(50.0)	P=0.537
	Secondary school	32 (44.4)	40 (55.6)	72 (31.3)	
	High school & above	6 (40.0)	9 (60.0)	15 (6.5)	
Job position	Cook	32(43.8)	41(56.2)	73 (31.7)	χ2=2.907,
	Waiter	48(48.5)	51(51.5)	99 (43.1)	P =0.234
	Cleaner	34(58.6)	24(41.4)	58 (25.2)	
Service years	<2	39(67.2)	19(32.8)	58 (25.2)	χ2=13.73,
	2-4	43(49.4)	44(50.6)	87 (37.3)	p=0.008
	5-7	18(45.0)	22(55.0)	40 (17.4)	
	8-10	8(32.0)	17(68.0)	25 (10.9)	
	>10	6(30.0)	14(70.0)	20 (8.7)	
Wears appropriate outer coat	Yes	77(47.0)	87(53.0)	164(71.3)	χ2=1.562,
	No	37(56.1)	29(43.9)	66(28.7)	P=0.211
Cleanness of outer garments	Kept	49(40.8)	71(59.2)	120(52.2)	χ2=7.653,
	Not kept	65(59.1)	45(40.9)	110(47.8)	P=0.006
Hand washing habit after toilet	With soap and water	83 (46.9)	94(53.1)	177 (77.0)	χ2=2.195,
	With water only	31 (58.5)	22 (41.5)	53 (23.0)	P=0.138
Hand washing habit after touching dirty materials	With soap and water	60 (45.9)	72 (54.5)	132 (57.4)	χ2=2.094,
	With water only	54 (55.1)	44 (44.9)	98 (42.6)	P=0.148
Hand washing habit before handling food	With soap and water	98 (48.8)	103 (51.2)	201 (87.4)	χ2=0.417,
	With water only	16 (55.2)	13 (44.8)	29 (12.6)	P=0.518
Finger nail status	Trimmed	87 (47.0)	98(53.0)	185(80.4)	χ2=2.437,
	Semi-trimmed	27 (60.0)	18 (40.0)	45(19.6)	P=0.119
Medical checkup	Checked	59 (45.0)	72 (55.0)	131 (57.0)	χ2=2.495,
	Not checked	55 (55.6)	44 (44.4)	99 (43.0)	P=0.114
Presence of rings	Observed	29(50.0)	29(50.0)	58(25.2)	χ2=0.006,
	Not observed	85(49.4)	87(50.6)	172(74.8)	P=0.939
Hygiene training	Trained	73 (45.6)	87 (54.4)	160 (69.6)	χ2=3.265,
	Not trained	41 (58.6)	29 (41.4)	70 (30.4)	P=0.071

CHAPTER SIX: DISCUSSION

Food handlers are an important vehicle for microorganisms, and improper handling practices may cause food contamination and consequently food borne diseases, which pose a potential risk to public health [27]. This study is taken to assess bacterial hand contamination among food handlers working in the student cafeterias of Jimma University main campus.

In this study 49.6% participants' hands were contaminated with one or more potentially food borne bacterial contaminants. *Staphylococcus aureus* 23.5%, *Klebsiella species* 16.1%, *E. coli* 0.9%, *Enterobacter species* 9.1%, *Citrobacter species* 4.3%, are among the most commonly isolated bacterial hand contaminants. The overall hand contamination rate of enteric bacterial contaminants was 31.7% among the participants. Similar types of bacterial contaminants were identified in Gondar [49], Nigeria [45], Egypt [43], Iran [36], Brazil [40] and Turkey [39].

Toxin-producing strains of Staphylococcus aureus are the leading cause of gastroenteritis following handling of food by persons who carry this bacterium in their noses and skin [47]. The present study *Staphylococcus aureus* were isolated from 23.5% food handlers' hands. This figure is comparable to 16.5%, 17.5%, and 31% prevalence reported in Gondar, Saudi Arabia, and Egypt respectively [49, 44, 43]. However, it is higher than 12.6%, and 7.1% incidence reported in Iran [36], and Nigeria [45] respectively. Nevertheless, the finding of the current study is lower than 42%, and 70% prevalence reported in Mexico [41], and Turkey [39] respectively. The discrepancy in socioeconomic status, type of food establishment, and lack of personal hygiene may explain this difference. The Isolation of *Staphylococcus aures*, reflect improper hygiene practices such as pocking fingers into the nose.

Enteric pathogens that are believed to be capable of being transmitted by food workers include, but are not limited to, *E. coli, Salmonella spp., Shigella spp.* In addition, pathogens such as *Proteus,* and *Klebsiella*, which could originate from raw animal products, can contaminate hands from where they could be transferred to foods, equipment and other workers [15].

Enteric pathogens are among the transient hand flora that can be easily removed by hand washing. Isolation of these organisms includes a faeces-to-hand spread and indicating a poor hygiene practices of the food handlers [38]. Their presence indicates fecal contamination and

poor hygiene practices food handlers are not taking enough care of hand hygiene [39]. In the present study' Enterobacteriaceae were identified from hands of 31.7% food handlers'. This result is nearly comparable to 38% isolation rate reported in Mexico [41], and higher than 6.9% reported in Egypt [43]. However it is lower than 44%, and 55.6% reported in South Africa [20], and Brazil [27] respectively. This could be resulted due to difference in source population, and type of food establishment. Isolation of Enterobacteriaceae from hands reflects contamination with fecal matter, and inadequate and poor hand washing habit which may pose potential risk of food borne outbreaks.

E. coli is naturally found in the human intestine and although most strains are harmless, some serotypes 0157:H7 can cause serious illness [46]. *E.coli* is normally absent from hands and the presence of *E.coli* gives a better indication of recent fecal contamination with enteric pathogens [20]. *E.coli* was detected on the hands of 10.9% of food handlers' in the current study, which is in line with 7.8%, and 6.8% carriage reported in Turkey, and Brazil respectively [39, 40]. However, it is lower than 22% carriage reported in Iran [36]. Nevertheless, this figure is higher than 3.9%, 3.1%, 2.5%, and 1.8% isolation rate reported in Turkey [25], Gondar [49], Saudi Arabia [44], and Nigeria [45] respectively. The difference between our results and the previous studies may be attributed to sampling techniques.

In our study, no significant association was found for bacterial hand contamination by sex, educational background, medical check-up, training status, hand washing habit, and fingernail status of food handlers. However, there was significant association between bacterial hand contamination rate and service years (Chi-square=13.732, DF=4, P<0.05). This result indicated that food handlers more work experience have less risk of bacterial hand contamination. This could be explained as food handlers with more work experience have better personal hygienic practices than inexperienced food handlers. There is a significant difference in bacterial hand contamination rate among different age groups ($\chi2$= 11.308, DFP=3, P=0.010). This can be explained as younger food handlers have poor hygienic practices. The bacterial hand contamination rate has significant association Cleanness of outer garments ($\chi2$=7.653, DF=1, P=0.006). Undoubtedly, in-depth training about personal hygienic practices of new employees, inexperienced, and young food handlers could minimize the effect of service on bacterial hand contamination rates

Persons working in food services have to go through periodic medical examination. The interview result of our study showed that only 56.7% of food handlers had taken medical checkup. This figure is comparable to 63.2% reported in Mekele University [32]. However, it is much higher than 22.7% reported in Mekele [33], and the result reported in Bahirdar in which none of the participants come across regular medical examinations [30]. The difference with respect to medical checkup can be explained by better provision and enforcement in Jimma University.

It is known that improper handling is one of the main causes of food borne disease and that inappropriate hand hygiene is an important risk factor for food contamination [27]. Food handlers should always wash their hands when their level of cleanliness may affect food quality; for example: just before food handling, after any interruption, after touching contaminated material, after using the bathroom and whenever else needed. They should not smoke, sneeze, spit, cough, eat, handle money or engage in any act that could contaminate the food during the performance of their activities [23].

Hygienic assessment of the food handlers revealed that 77% of food handlers have a habit of hand washing with soap and water after toilet, while others used only water. This figure is nearly similar to 70.4%, and 89% reported in Mekele, and Gondar respectively [32, 49]. However, it was lower than 90.6%a reported in Bahirdar [30]. In the present study only 57% of food handlers have hand washing habit after touching dirty materials, and different body parts such as nose. This result shows food handlers negligence, and lack of awareness on sources of food contamination.

Food handlers should receive training before starting work in any food establishment, with a periodic refreshing training [31]. In this study 68.7 % food handlers have got short course of training on food hygiene. This figure is much higher than 14%, and 12.3% reported in Bahirdar, and Mekele respectively [30, 32]. This gap can be due to both studies enumerated only certified trainings. However, in the current study food handlers have got only short course of food safety training organized by the student cafeteria office. None of the food handlers were certified by formal training. Effective training of food handlers, may lead to an improvement in hygienic practices.

Food handlers should cover hair and wear appropriate protective covering , cut their fingernails short and during handling they should remove jewelry from their hands[23]. In the present study, 164 (71.3%) food handlers were observed wearing outer working Coat, while only 40.4% had worn hair net. This result is in line with the report a cross-sectional study in mekele in which 72.6% of the food handlers were found wearing outer working garments, and 39% had worn hair net [33]. Nevertheless, it is higher than the figure reported in Ambo in which only 28% of food handlers' worn outer garment and hair covers [28]. This gap might be due to differences in socio-economic condition.

Moreover, in our study 80.4% of food handlers' fingernails were trimmed. This figure is by far comparable to 76.2%, and 88.4% reported in Mekele town [33], and Mekele University [32] respectively. However, it is higher than 70% reported in Ambo [28]. Even though it had no association with the isolation rate of potential bacterial pathogens in this study, fingernails can serve as a vehicle for transport of microorganisms from their source to the foods or/and directly into the body. Beside this, 25.2% food handlers have worn finger ornaments. This figure is similar to 27.1% reported in Ambo [28], and lower than 35.7% reported in Mekele [33].

CHAPTER SEVEN: LIMITATIONS OF THE STUDY

As far as the study design is cross sectional, it simply provides information about relationship between the dependent and independent variables. Beside this Total Plate Count and Colliform count were not done because of resource and time constraint. Serological identification of *Salmonella species* was not carried out. Antimicrobial susceptibility test for potential food borne bacterial contaminants was not done. Moreover, there is a scarcity of studies focused on isolation of bacteria from hands. This fact makes difficult the comparison of our results with that found by other researchers especially from developing countries.

CHAPTER EIGHT: CONCLUSION AND RECOMMENDATIONS

8.1. CONCLUSION

In conclusion, our findings showed 114 (49.6%) carriage of potential food borne bacterial hand contaminants and 73(31.7%) were tested positive for enteric bacterial hand contaminants. The following food borne bacterial hand contaminants were isolated with the corresponding prevalence rate: *S. aureus* 54(23.5%), *Klebsiella spp.* 37(16.1%), *E.coli* 25(10.9%), *Enterobacter spp.* 21(9.1%), *Citrobacter spp.* 10(4.3%), *Serratia marcescens* 6 (2.6%), *Pseudomonas aeruginosa* 8(3.5%), *Proteus spp.* 5(2.2%), *Providencia rettegri 3(1.3), and salmonella spp.*2 (0.9%). These findings emphasized the importance of food handlers hand as potential vector of food borne bacterial pathogens that could constitute a potential risk of food borne disease outbreaks. Bacterial hand contamination rate have significant association with service years (Chi-square=13.732, DF=4, P=0.008), age ($\chi2$= 11.308, P=0.010) and cleanness of outer garments ($\chi2$=7.653, P=0.006). Despite short course of informal food hygiene training none of the participants has been certified by formal training.

8.2. RECOMMENDATION

Based on the findings of the study the following recommendations are made:

➢ As a responsible body to JU:

- Instruction regarding proper methods of hand washing should become a part of new employees, as well as young and inexperienced food handlers' orientation, education.

- Food handlers should be well trained about personal hygienic practices pointing out on importance of hand hygiene and cleanness of outer garments

- Close follow up, and regular supervision of personal hygienic practices of food handlers should be used as controlling strategies

➢ Future studies should focus on enumeration of bacterial hand contaminants, and assessing sanitary facilities of the working environment.

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ANNEXES

Annex-I: Information sheet, and consent form for Study participants

This study is aimed at Assessing bacterial hand contamination among food handlers working in the student cafeterias of Jimma University main campus, Jimma, south west Ethiopia.

We are requesting you and others to voluntarily participate in this study. What we expect from everyone is to be examined for bacterial hand carriage, as well as to answer a few questions regarding risk factors. The laboratory examination involves collection of hand rinse samples that should be collected using a sterile plastic bag containing sampling solution.

Giving hand rinse sample doesn't have any harm to your health and any other aspects like your job rather you will be benefited. That is, if there is a positive finding for potential bacterial pathogen in laboratory examination, we will do stool culture diagnosis for you. In case of positive stool culture we will communicate you with the university's administration to provide treatment and health education.

Any information that we collect about you during this research will be kept in secret. Information about your identity will be put away after re-coding your file; and kept in a secured place. Only the principal investigator will be able to link your identity with the code number.

Since participation in this study is entirely voluntary, you can refuse to participate in this study at any time. Your refusal to participate will not affect any of your benefits. Any information that we collect about you during this research will be kept in secret.

Contact address

If you have any question and in case of urgency you can contact:-

Tsegaye Assefa Tel: - 0910210514

Jimma University, faculty of Medical sciences and public health, School of Medical Laboratory Sciences and Pathology, department of Medical Microbiology (P.O. Box- 378, Jimma, Ethiopia Tel. 0471120945)

Consent form

I have read the information sheet (or it has been read to me); I have understood that it involves the study about assessment of bacterial hand contamination of food handlers working in the student cafeterias Jimma University main campus. And also I have cleared about the Purpose of the study, procedure to be carried out, Risks associated with the study, benefits of the study and Confidentiality of the information.

I, the undersigned, confirm that, as I give consent to participate after a clear understanding of the objectives and conditions of the study & with recognition of my right to withdraw from the study if I change my mind.

I …………………………………………………….do interestingly give consent to Mr./Mrs./Miss …………………………..to include me in the proposed research. The proposal has been explained to me in the language I understand.

Annex-II: Questionnaire

Questionnaires for data collection on entitled "Assessment of bacterial hand contamination among food handlers working in the student cafeterias of the of Jimma University main campus, Jimma, southwest Ethiopia"

Serial number: _____

Name of the student cafeteria _____ Establishment code No._____

General instruction

Almost all of the questions and observational checklists have a precoded response. So it is important to follow the following instructions while you are interviewing the respondents and recording their responses

Ask or read each questions exactly as written on the questionnaire

Circle the responses that best match with the answer of the respondent

Do not read the precoded responses for the respondents, listen only the response of the respondents

For observational types go and observe each of the requested items and record your observations exactly on the pre-coded response formats

Part-I: General information

Sr.no	Question	Response	Code
101	Sex	1. Female 2.Male	
102	Age in years	_____	
103	Marital status	1. Single 2. Married 3. Divorced 4. widowed	
104	Ethnicity	1. Oromo 2. Amhara 3. Gurage 4. Others (specify)_____	
105	Religion	1. Orthodox 2. Muslim 3. Protestant 4. Others (specify)_____	
106	Educational background	1. No formal education 2. Elementary school 3. Secondary school 3. High school 4. Above	
107	Job position in the cafeteria	1. Cook 2. Waiter or serving 3. Cleaners 4.Others(specify)_____	
108	Service in years	_____	
109	Certified in food hygiene taining?	1. yes 2. Trained but not certified 3. No	
110	Hand washing habit after toilet?	1. Yes, with water and soap 2. Yes, only with water 3. No	
111	Hand washing habit after touching dirty materials and body parts (nose, hair, & etc) while handling foods	1. Yes, with water and soap 2. Yes, only with water 3. No	
112	Hand washing habit before food handling?	1. Yes, with water and soap 2. Yes, only with water 3. No	
113	Wear gloves while handling foods?	1. Yes 2. No	
114	Have you ever have a routine medical checkup in the last 6 month?	1. Yes 2. No	
115	Salary in birr	_____	

Part-II: Observational checklist for personal hygiene practice assessment

Sr.no	Question	response	code
201	Does the worker wear appropriate outer coat?	1. Yes 2. No	
202	Does the worker wear appropriate hair cover?	1. Yes 2. No	
203	Cleanness of outer garments during visit	1. Kept 2. Not kept	
204	Finger nail status	1. Trimmed 2. Semi trimmed 3. Not trimmed	
205	Wear any jewelry, or ring at time of visit?	1. Observed 2. Not observed	

This is the end of the Interview!!

Thank you for your cooperation!!

Annex-III: Sample collection and Laboratory investigation

A. Sample collection

Before starting any meal preparation activity including hand washing (if any), participant's hands will be sampled for microbial testing. No notification was given in advance, and no extra hand hygiene was allowed during the hand rinse sample collection. A sterile polyethylene plastic bag technique was employed to collect the hand rinse samples. Buffered peptone water (0.1% BPW) (Difco/Becton Dickinson, Franklin Lakes, NJ) was used as sampling solution.

Food handlers with rings are asked to worn out prior to specimen collection

Participants are asked to dip their hands into sterile polyethylene plastic bag containing 100 milliliter of buffered peptone water (BPW)

The bag is grasped tightly around the participant's wrist and the Peptone buffer is rubbed through the wall of the bag by the investigator for 1 min, over all surfaces of the participant's hands, particularly around the nails and palm.

The bag is immediately sealed and transported to the Jimma university medical microbiology laboratory and examination is carried out on the day the samples are collected.

B. Isolation and identification of food borne bacterial pathogens

All media used in this study were from Oxoid Ltd. and were prepared according to the manufacturer's instructions. The hand rinse samples were vortexed for one1 minute before microbiological examination was performed. The samples was separately spread-plated onto McConkey (for detection of enteric bacteria), MSA (for detection of *staphylococci*), and XLD (for detection of *Salmonella*, and *Shigella*). Rappaport vassiliadis (RV) was used as a primary enrichment for the identification of Salmonella and Shigella. The bacterial colonies grown on the agar media were presumptively identified by colonial morphology and gram staining and a battery of biochemical tests like reaction on oxidase, catalase, simmon citrate, indole production, urease, motility, coagulase, methyl red-voges proskaeur (MR-VP), LDC, KIA, gas and Hydrogen Sulfide (H_2S) production. The identified isolates were kept frozen at -70^0C in 15% glycerol broth.

For the isolation of S. *aureus*, a loop full of sampled broth was heavily plated on Mannitol Salt Agar (MSA) (Himedia, India) and incubated at 37°C for 48 h. The Gram reactions and cellular morphology (Mannitol fermenting golden yellow colonies) of isolates were determined. Presumptive Staphylococcus aureus isolates were tested for catalase activity, from colonies grown on NA, using 3% hydrogen peroxide on a glass slide and observing for vigorous bubbling.

For the isolation of Enteric pathogens and Pseudomonas a loop full of sampled broth was plated on MacConkey agar and incubated at 37°C for 24-48 h. Colonies on MacConkey agar were differentiated based on their characteristics to ferment lactose. Pink colour characterizes lactose fermenters whereas colourless colonies are non-lactose fermenters. Gram negative bacteria were further tested for their motility and characterized using arrays of biochemical tests including Oxidase, indole, urease, Krigler Iron agar (KIA), Simmon Citrate Agar, and Lysine Decarboxylase (LDC).

For the isolation of *Salmonella* and *Shigella*, a volume of 1 ml of hand rinse sample was inoculated in to 10 ml of Rappaport-Vassilidias (RV) broth (OXOID, Hampshire, UK) and incubated at 42°C for 24-48 h as primary enrichment. The broth was then sub-cultured onto Xylose Lysine Deoxycholate (XLD) agar (OXOID, Hampshire, UK). After 18-24 h of incubation at at 37°C, *Salmonell*a and *Shigella* were distinguished by their characteristic appearance on the XLD Agar and by using biochemical tests and observing their reaction on Urease, LDC, KIA and Simmon Citrate.

BIOCHEMICAL TESTS

Catalase test

This test is used to differentiate those bacteria that produce the enzyme Catalase such as staphylococci from non Catalase producing bacteria such as streptococci.

Principle: Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. An organism is tested for catalase production by bringing it in to contact with hydrogen peroxide. Bubbles of oxygen are released if the organism is a catalase producer. The culture should not be more that 24 hour old.

Material Required

- Hydrogen peroxide,

- 3% H_2O_2 (10 volume solution)

- Test tubes

Method

- Pour 2-3 ml of the hydrogen peroxide solution into a test tube

- Using a sterile wooden stick or a glass rod (not a nichrome wire loop), remove several colonies of the test organism and immerse in the hydrogen peroxide solution.

- Look for immediate bubbling.

Caution: Care must be taken when testing an organism cultured on a medium containing blood because catalase is present in red cells. If any of the blood agar is removed with the organism, a false positive reaction may occur.

Results

Active bubbling ------------------ Positive test (Catalase produced)

No release of bubbles ---------- Negative test (No catalase produced)

Note: if the organism has been cultured on an agar slope, pour about 1ml of the hydrogen peroxide solution over a good growth of the organism, and look for the release of bubbles. When the rapid slide technique is used, the hydrogen peroxide solutions should be added to the organism suspension after placing the slide in a petridish. The dish should then be covered immediately, and the preparation observed for bubbling through the lid.

Control

Positive catalase control – staphylococcus species

Negative catalase control – streptococcus species

Coagulase test

Principle

Coagulase causes plasma to clot by converting fibrinogen to fibrin.

Required

EDTA anticoagulated human plasma (preferably pooled and previously HIV and hepatitis tested) or rabbit plasma. The plasma should be allowed to warm to room temperature before being used.

Slide test method (detects bound coagulase)

1. Place a drop of distilled water on each end of a slide or on two separate slides.

2. Emulsify a colony of the test organism (previously checked by Gram staining) in each of the drops to make two thick suspensions.

Note: Colonies from a mannitol salt agar culture are not suitable for coagulase testing. The organism must first be cultured on nutrient agar. Suspensions, and mix gently. Look for clumping

3. Add a loopful (not more) of plasma to one of the organisms within 10 seconds.

No plasma is added to the second suspension. This is used to differentiate any granular appearance of the organism from true coagulase clumping.

Results

Clumping within 10 secs . . . . S. aureus

No clumping within 10 secs . . . . Other Staphylococcus species

Oxidase test

The oxidase test identifies organisms that produce the enzyme cytochrome oxidase. Cytochrome oxidase participates in the electron transport chain by transferring electrons from a donor molecule to oxygen. The oxidase reagent contains a compound that changes color when it becomes oxidized. If the test organism produces cytochrome oxidase, the colorless reagent used in the test will detect the presence of the enzyme oxidase and, reacting with oxygen, turn violet to purple. The oxidase test is a key test to differentiate between the families of Pseudomonadaceae (oxidase positive) and Enterobacteriaceae (oxidase negative).

Principle

- A piece of filer paper is soaked with a few drops of oxidase reagent.

- A colony of the test organism is then smeared on the filter paper. Alternatively an oxidase regent strip can be used.

- When the organism is oxidase-producing, the phenylenediamine in the reagent will be oxidized to a deep purple color..

Material Required

- Fresh Oxidase reagent (Tetramethyle-p-phenylenediamine dihydrochloride, 1%)

- Filter paper or oxidase regent strip

Note:

- Fresh oxidase reagent is easily oxidized,

- when oxidized it appears blue and must not be used.

Method

- Place a piece of filter paper in a clean petridish

- add 2 or 3 drops of freshly prepared oxidase reagent,

- Using a piece of stick or glass rod (not an oxidized wire loop), remove a colony of the test organism and smear it on the filter paper.

- Look for the development of a blue-purple color within a few seconds.

Result

- Blue – purple color ……..positive Oxidase test (Within 10 seconds)

- No blue – Purple color …Negative Oxidase test (Within 10 seconds)

Note: Ignore any blue – purple color that develops after 10 seconds.

Method using an oxidase regent strips

- Moisten the strip with a drop of sterile water.

- Using a piece of stick or glass rod (not an oxidized wire loop) remove a colony of the test organism and rub it on the strip.

- Look for a red-purple color within 20 seconds.

- Red-purple color………….positive oxidase tests.

Controls

- Positive oxidase control: Pseudomonas aeruginosa

- Negative oxidase control: Escherichia coli

Indole test

The test detects the ability of an organism to produce Indole from Tryptophan present in the medium. Testing for Indole production is important in the identification of enterobacteria. Most

strains of *E. coli, P.vulgaris, P.rettgeri, M.morganii*, and *Providencia* species are Indole positive organisms.

Principle: The test organism is cultured in a medium which contains tryptophan. The enzyme tryptophanase can convert the amino acid, tryptophan, to indole, ammonia, and pyruvic acid. Indole production is detected by Kovac's or Ehrlich's reagent which contains 4(p)-dimethylamino-benzaldehyde. When Kovac's reagent, which contains hydrochloric acid and dimethylaminobenzaldehyde and amyl alcohol, a red layer form when indole is present. No color in this layer is a negative result. Kovac's reagent is recommended in preference to Ehrlich's reagent for the detection of indole from enterobacteria.

Materials required

- Kovac's or Ehrlich's reagent

- bijou bottle/test tube

Method

An indole test can be performed:

- As a single test using tryptone water and kovac's reagent.

- As a combined beta-glucuronidase-indole test using a Rosco PGUA/indole identification tablet and kovac's reagent. This is useful when identifying *E. coli*.

- As a combined lysine decarboxylase-indole test using a Rosco LDC/indole identification tablet. This is useful in helping to identify *salmonellae* and *shigella*

Indole test using tryptone water and kovac's reagent

- Inoculate the test organism in a bijou bottle containing 3 ml of sterile tryptone water.

- Incubate at $35 - 37^o$C for up to 48 hr

- Test for indole by adding 0.5ml of Kovac's reagent.

- Shake gently.

- Examine for a red color in the surface layer within 10 minutes.

Results

Red surface layer……………………… Positive indole test

No re surface layer……………………. Negative indole test

Control

Positive control ……………………… *Escherichia coli*

Negative control………………………. *Klebsiella pneumoniae* .

Citrate utilization test

The test detects the ability of an organism to use citrate as its only source of carbon. This test is one of several techniques used occasionally to assist in the identification of enteric bacteria.

Principle: Some bacteria can obtain energy in a manner other than by the fermentation of carbohydrate by using citrate as source of carbon. The utilization of citrate by a test bacterium is detected in citrate medium by the production of alkaline by-products. The medium includes sodium citrate as the sole source of carbon and ammonium phosphate as the sole source of nitrogen. Bacteria that can use citrate can also extract nitrogen from the ammonium salt, with the production of ammonia (NH^+), leading to alkalinization of the medium. In the presence of the indicator Bromthymol blue the medium will be converted from green (at pH 6.0) to blue (at a pH above 7.6).

Materials required

- Simmon's citrate medium/agar, and inoculating loop

Method

- Prepare slopes of the medium in bijou bottles as recommended by the manufacturer (store at 2-8 ^{0}C)

- Using a sterile straight wire, first streak the slope with a saline suspension of the test organism and then stab the butt.

- Incubate at 35 ^{0}C for 48 hours

- Look for a bright blue color in the medium

Results

Bright blue…………………………………. Positive citrate test

No change in color of medium……………. Negative citrate test

Controls

Positive control……………………………… *Klebsiella pneumoniae*

Negative control………………………….. *Escherichia coli*

MRVP (methyl red-Vogues Proskauer) test

This test is used to determine two things. The MR portion (methyl red) is used to determine if glucose can be converted to acidic products like lactate, acetate, and formate. The VP portion is used to determine if glucose can be converted to acetoin. These tests are performed by inoculating a single tube of MRVP media with a transfer loop and then allowing the culture to grow for 3-5 days. After the culture is grown, about half of the culture is transferred to a clean tube. One tube of culture will be used to conduct the MR test, the second tube serves as the VP test.

A. Methyl red (MR) test:

- Methyl red is added to the MR tube.

- A red color indicates a positive result: Glucose can be converted into acidic end products such as lactate, acetate, and formate.

- A yellow color indicates a negative result: Glucose is converted into neutral end products.

B. VP (Vogues Proskauer) test:

- First alpha-napthol (also called Barritt's reagent A) and then potassium hydroxide (also called Barritt's reagent B) are added to the VP tube.

- The culture should be allowed to sit for about 15 minutes for color development to occur.

- If acetoin is produced then the culture turns to red color (positive result);

- If acetoin is not produced then the culture appears yellowish in color (a negative result).

- ✓ VP (Vogues Proskauer) test positive bacteria include: *Klebsilla* spp, *Enterobacter* spp and *Serratia* spp, *Eltor vibrio*, *Staphylococci*

- ✓ Methyl Red positive bacteria include: *Escherchia* spp., *Citobacter* spp., *Salmonella* spp, *Proteus* spp., *Yersinia* spp., *Staphylococci* Etc

- ✓ MR-VP test positive bacteria include: *Staphylococci*

Krigler Iron Agar (KIA)

Principle: both the butt and the slant were streaked, to determine fermentation of glucose, lactose and to see the production of hydrogen sulfide. Shigella and salmonella species characteristically produce an alkaline (red) slant and an acid (yellow) butt, little or no gas, and with no or with Hydrogen sulfide, respectively.

- KIA is a composite medium containing glucose, lactose, phenol red and ferric citrate.

- A yellow base indicates glucose fermentation

- A yellow base and slope indicates both glucose and lactose fermentation.

- Bubble in the medium indicate gas production from glucose

- Blackening of the medium indicate H_2S production

Motility test

To determine whether the organism is motile or not it was stabbed with a straight inoculating needle, making a single stab about 1–2 cm down into the medium. Motility is indicated by the presence of diffuse growth (appearing as clouding of the medium) away from the line of inoculation.

- The motility test is not a biochemical test since we are not looking at metabolic properties of the bacteria.

- Rather, this test can be used to check for the ability of bacteria to migrate away from a line of inoculation.

- To perform this test, the bacterial sample is inoculated into motility media using inoculating wire.

- Simply stab the media in as straight a line as possible and withdraw the needle very carefully to avoid destroying the straight line.

- After incubating the sample for 24-48 hours, observations can be made.

- Check to see if the bacteria have migrated away from the original line of inoculation.

- If migration away from the line of inoculation is evident then you can conclude that the test organism is motile (positive test).

- Lack of migration away from the line of inoculation indicates a lack of motility (negative test result).

Table: Interpretation of biochemical tests for some Enterobacteria and P. aeruginosa

Parameters	Pure isolates									
	I	II	III	IV	V	VI	VII	VIII	IX	X
Lactose	A	_	A	_	A	A	A	_	_	_
Oxidase	_	+	_	_	_	_	_	_	_	_
Indole	+	_	_	+_	_	_	_	_	d	+
Citrate	+	+	+	_	+	+	_	+	_	+
VP	_	_	+	_	_	+	+	_	_	_
LDC	+	_	+	_	_	+	d	+_	_	_
H$_2$S	_	_	_	+	_	_	_	+	_	_
Gas	+	_	+	+	+	d	+	+	_	d
Motility	_	_	_	+	+	+	+	+	_	+
Urease	_	_	+	+	d	_	+	_	_	+
Methyl Red	+	+	+	+	_	+	+			
Glucose	A	_	A	A	A	A	A	+	+	+
Sucrose	A	_	A	A	d	_	A	_	_	d
Most Propable Bacteria	Ecoli	Pseu	Kleb	Prot	Citro	Sera	Entr	Salm	Shig	Prov

+ = positive , -- = negative , A = Acid production, d= variable, Staph=Staphylococcus spp, E.coli=E. coli, Pseu=Pseudomonas aeroginossa , Kleb= Klebsiella spp , Prot= Proteus spp ,Citro= Citrobacter spp, Sera= Serratia marcescens, Entr= Enterobacter spp, Salm= Salmonella spp, Shig= Shigella spp, Prov= Providencia rettegri
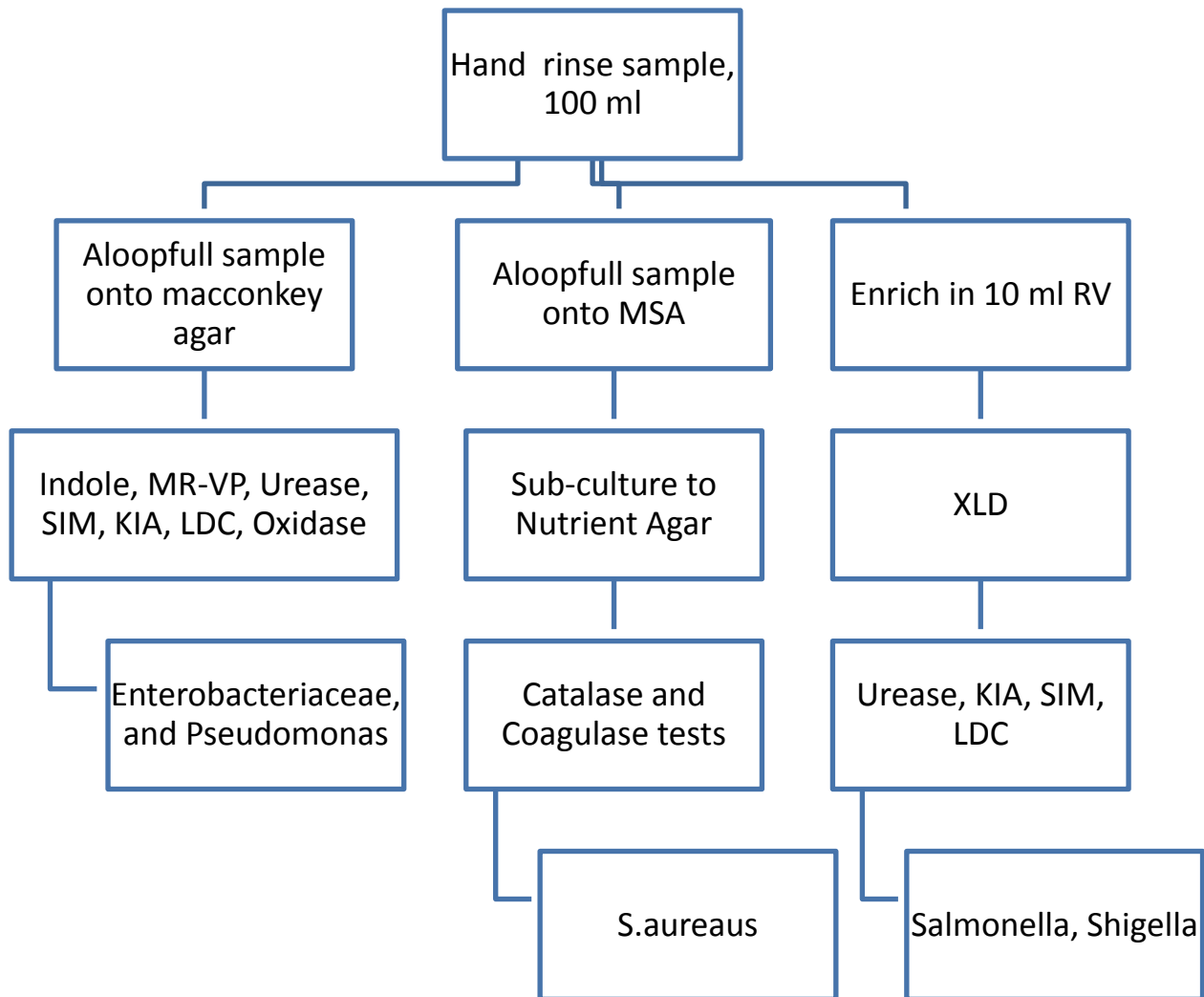
Figure: Simplified laboratory flow chart for identification of potential food borne bacterial hand contaminants