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ORIGINAL ARTICLE

Screening Of *Staphylococcus Species* From Beef, Mutton, Fish And Quail Meat Samples Collected from Different Localities of Lahore

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ABSTRACT

Food-borne bacteria have been the most concern in public health and food safety. These bacteria lead to the nosocomial infections. Foodstuff contamination may occur directly from infected food-producing animals or may result from poor hygiene during production processes, or the retail and storage of foods, since humans may carry the microorganism. Staphylococcus food poisoning is one of the most economically important food-borne pathogen worldwide. This study aimed to determine the presence of Staphylococcus on food-contact surfaces in meat and seafood environments and identify co-existing micro biota has been therefore carried out. From the collected samples, pathogenic specie of bacteria Staphylococcus was isolated and identified by growth in its selective media using microbial techniques. The external surfaces of exposed meats that are present on different shops are responsible for the transmission of the infectious diseases by pathogenic bacteria. The results shows that meat products may act as an important vehicle of transmission for well-established pathogens and this is due to unhygienic conditions of the environment and can cause a danger to the public health when they are touched by these contaminated meats. So it is necessary to do proper washing and avoid precooked meat for eating purposes.

Keywords*: Example (Staphylococcus, Selective media, Hygienic, microbial techniques)*

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INTRODUCTION

Coagulase-Negative *Staphylococcus* e.g. *Staphylococcus aureus* usually found on skin or in the nasal environment and only survives on dry skin on the outside of the body. In our study Gram positive bacteria were more frequently isolated from all surfaces compared to Gram negative. This could be in part due to the fact that survival of Gram +ve species on laminate surfaces is greater than that of Gram negative organisms. However, Gram +ve and Gram –ve bacteria have been shown to have similar transfer rates from laminate surfaces to fingertips [1]. Normal skin is inhabited with two categories of bacteria: transient and resident. Resident floras, which are attached to deeper layers of the skin, are more resistant to removal by routine washing. Coagulase-negative staphylococci and Gram +ve diphtheroids are members of this group. On the other hand, transient flora colonizes the superficial layers of the skin, and is more amenable to removal by routine hand washing [2].

Bacteria that are often found in a healthcare environment include coagulase-negative *Staphylococcus*, *Bacillus* species, Corynebacterium species, *streptococci*, *Clostridium perfringens*, *Enterococcus* species, *Staphylococcus aureus*, gram negative bacteria, and fungi [3]. Of significant importance in healthcare environments involve antibiotic resistant strains of microbes which include *Staphylococcus aureas*, Vancomycin-resistant enterococci, and methicillin-resistant *Staphylococcus aureas* (Methicillin Resistant *Staphylococcus aureus*). The capability of these bacteria to survive for more than 24 hours further increases their chances of contamination in other places.

S. aureus colonizes in 30% to 50% of healthy human population [4], and the anterior nares of the nose are the most frequent carriage site for the bacteria [5]. In the National Health and Nutrition Examination Survey conducted in 2001-2002 in the United States, it was estimated that nearly one third (32.4%) of the non-institutionalized population including children and adults were nasal carrier [6]. Prevention of staphylococcal food poisoning from the infected food handlers may be difficult as carriers are asymptomatic [7, 8]. Other studies also reported high prevalence of enterotoxin-producing *S. aureus* in food handlers. A cross-sectional study conducted among 127 food handlers working in cafeterias in

Ethiopia indicated that 16.5% of fingernail contents of the food handlers were cultured positive for *S. aureus* [9]. Another study done in Botswana reported that an even higher proportion (57.5% out of 200 food handlers) was tested positive for *S. aureus* [10]. *S. aureus* is also present in food animals, and dairy cattle, sheep and goats, particularly if affected by subclinical mastitis, are likely contaminants of milk [11]. Air, dust, and food contact surfaces can also serve as vehicles in the transfer of *S. aureus* to foods.

EXPERIMENTAL

Material and Methods: The following materials were utilized for the current research work: Nutrient agar (NA), produced by Oxoid limited, Basingstoke Hampshire England, Nutrient broth (NB), produced by Oxoid limited, Basingstoke Hampshire England, Mannitol salt agar (MSA), produced by Hardy diagnostic Criterion, Chicago, USA, Blood agar (BA), produced by Hi media laboratories, Pennsylvania, United States, Chromagar staph (CAS), produced by BD diagnostic company, Germany, Europe, Phenylethyl alcohol agar (PEA), produced by Oxoid limited, Basingstoke, UK, Tryptic soy agar (TSA), produced by Oxoid limited, Basingstoke Hampshire England, Alcohol, Crystal violet, Iodine, Acetone- Alcohol and Safranine, produced by BD diagnostic company, stains and reagents, Germany, Europe

General procedure:

CULTURING IN NUTRIENT BROTH: Mix the appropriate amount of nutrient broth in distilled water and pour it in test tube at the marked level. Seal the mouth of test tubes by plugging and autoclave them. After autoclaving inoculate some test tubes with desired sample and place them on the shaker for overnight. Observe the results and note them.

CULTURING ON NUTRIENT AGAR: Make the appropriate amount of nutrient agar in conical flask. Then pour them in petri plates and allow them to solidify. Place them in incubator for overnight. Next day inoculate the sample by streaking it with red hot inoculating loop. Again place them in incubator for overnight to observe the growth of bacteria.

CULTURING ON MANNITOL SALT AGAR: Take 108g of MSA in 100ml distilled water and autoclaved it. Pour it in petri plates and place them in incubator. Take the inoculum from nutrient agar plates with sterile loop and streak it on MSA plate and place it overnight for the growth of bacteria. Only *S. aureus* can grow on this media. Because *S. aureus* can with stand high salt concentration. It is the selective media for the growth of *S. aureus.*

CULTURING ON BLOOD AGAR:

Take 42g of BA in 100ml distilled water and autoclaved it. Pour it in petri plates and place them in incubator. Take the inoculum from nutrient agar plates with sterile loop and streak it on BA plate and place it overnight for the growth of bacteria. Only *S. aureus* can grow on this media. This media supports the growth of fastidious bacteria such as *staphylococci* and *streptococci* because blood is the important ingredient of this media and it provides nutrients for the growth of bacteria.

CULTURING ON PHENYL ETHYL ALCOHOL AGAR: Take 40.5g of PEA in 100ml distilled water and autoclaved it. Pour it in petri plates and place them in incubator. Take the inoculum from nutrient agar plates with sterile loop and streak it on PEA plate and place it overnight for the growth of bacteria. Only *S. aureus* can grow on this media. This media supports the growth of fastidious bacteria such as *staphylococci* and *streptococci* because blood is the important ingredient of this media and it provide nutrients for the growth of bacteria. 5% blood is also present in this media and the morphology of colonies on this media is similar to that on BA media plate. It supports the growth of gram +ve bacteria.

CULTURING ON CHROMAGAR STAPH: CHROM agar *Staph aureus* is a selective and differential medium that has been shown to increase the recovery of *S. aureus* from clinical specimens. CHROM agar *Staph aureus*is a chromogenic medium, one of several chromogenic formulations offered by BD Diagnostic Systems. The enrichment base of nutritive agar supports the growth of microorganisms. The addition of a proprietary chromogen mixture differentiates the bacteria on the basis of color reactions. *S. aureus* colonies produce a distinctive mauve pigmentation, while coagulase-negative staphylococci appear as white, beige or light blue colonies. Other organisms are inhibited or grow poorly on the CHROM agar medium due to the presence of antimicrobial agents. About 80g of CHROM agar in mixed in 100ml distilled water and autoclaved media is poured in sterile petri plates and streak them and observe the growth of bacteria.

CULTURING ON TRYPTIC SOY AGAR: Tryptic Soy Agar supported excellent growth of aerobic and anaerobic microorganisms. It is a nutritious base and a variety of supplements are added to enhance the medium. Add 33g of medium in one litre of distilled water in a beaker and mix with the help of stirrer. After complete dissolve of media into the water, poured the media into the conical flask. Covered the mouth of the conical flask with the cotton plug and aluminum foil and autoclaved it. After 24 hours of incubation , streak these plates of TSA with the help of inoculating loop with the culture grown in the nutrient broth and were placed as inverted in the incubator for 24 hours at 45ºC.

GRAM STAINING: Wash the slide with water and dried with the help of filter paper. With the help of sterile inoculating loop, pick the bacterial culture and mix properly with one drop of water. Prepare the smear of the culture and fix it by passing one or two times over the flame. On the fixed smear, flood the crystal violet for one minute. Pour off excess dye and wash gently in tap water and drain the slide against a paper towel. Expose the smears to Gram's iodine for one minute by washing with iodine, then adding more iodine and leaving it on the smear until the minute is over. Wash with tap water and drain carefully. Wash with 95% alcohol for 30 seconds. Wash with tap water at the end of the 30 seconds to stop the decolorization. Counter stain with 0.25% safranin for 30 seconds. Wash, drain, blot, and examine under oil.

RESULTS AND DISCUSSION

NUTRIENT BROTH GROWTH:

MANNITOL SALT AGAR GROWTH:

BLOOD AGAR GROWTH:

PHENYL ETHYL ALCOHOL AGAR GROWTH:

CHROMAGAR STAPH GROWTH:

TRYPTIC SOY AGAR GROWTH:

DISCUSSION

The main purpose of this bacteriological study was to separate and detect the pathogenic microorganisms on the different food types such as meat to create public alertness about the health menaces resulting from these pathogenic microorganisms. To conduct this study, total 400 samples were collected from different places located in Lahore, Pakistan. All the samples were first grow on nutrient broth and nutrient agar, which ensured the presence of certain pathogenic microorganisms like *E.coli, Salmonella, Staphylococcus, Streptococcus*. For further confirmation and identification, the culture from the nutrient agar was streaked on different selective media and the presence of pathogenic bacteria ie *Staphylococcus aureus* was confirmed.

MSA is a differential and selective media. It is selective because its high salt concentration (7.5 %) inhibits the growth of most bacteria. However, *Staphylococcus* is able to tolerate this high salinity. MSA is differential because it contains the sugar mannitol and phenol red, a pH indicator. When mannitol is fermented, acid products are produced and the pH drops. Phenol red is yellow in color below pH 6.8. Thus, mannitol fermenters such as *Staphylococcus aureus* will have a yellow halo around them. Mannitol non fermenters such as *Staphylococcus epidermidis*will leave the MSA media unaltered (pink).

Blood agar plate (BAP) contains mammalian blood (usually sheep or horse), typically at a concentration of 5–10%. BAP are enriched, differential media used to isolate fastidious organisms and detect hemolytic activity. β-hemolytic activity will show lysis and complete digestion of red blood cell contents surrounding colony. γ-hemolysis (or *non-hemolytic*) is the term referring to a lack of hemolytic activity.

It contains meat extract, tryptone, sodium chloride, and agar. It ismedium that can distinguish normal from pathogenic bacteria based on the effect of bacterial hemolytic exotoxins on red blood cells.

Phenylethyl alcohol agar (PEA) is a selective medium used to cultivate Gram positive organisms. The active ingredient, phenylethyl alcohol, inhibits or markedly reduces growth of Gram negative organisms by interfering with DNA synthesis. PEA also prevents *Proteus* species from swarming across the surface of the agar. Phenylethyl Alcohol Blood Agar will not provide complete information for the identification of bacterial isolates. Additional test procedures and media are required for complete identification.

CHROM agar *Staph aureus* supply specially selected peptones nutrients. The addition of selective agents inhibits the growth of gram-negative organisms, yeast and some gram-positive cocci. The chromogen mix consists of artificial substrates (chromogens), which release an insoluble colored compound when hydrolyzed by specific enzymes. This facilitates the detection and differentiation of *S. aureus* from other organisms. *S. aureus* utilizes one of the chromogenic substrates, producing mauve-colored colonies. The growth of mauve-colored colonies at 24 h is considered positive for *S. aureus* on CHRO Magar *Staph aureus*. Bacteria other than *S. aureus* may utilize other chromogenic substrates resulting in blue, blue-green, or if no chromogenic substrates are utilized, natural colored colonies.

Tryptic Soy Agar (TSA) facilitated vigorous growth of aerobic and anaerobic microorganisms. TSA, a general purpose medium, is commonly referred to as Soybean-Casein Digest Agar. TSA is a nutritious base, and a variety of supplements can be added to enhance this medium. The addition of 5% sterile, defibrinated sheep, horse, or rabbit blood provides an excellent nonselective medium, used to determine hemolytic reactions of bacteria.

The circumstances mentioned above clearly indicate that contaminated meats are the energetic source of spreading different pathogenic microorganisms to humans and causes number of serious diseases. The main objective of the present study was to raise the alertness among the people about the lethal effects of not properly washed or precooked meats and the pathogenic microorganisms. The presence of these deleterious bacteria makes our environment unhealthy.

Environment of Pakistan is favorable for the growth of pathogenic microorganisms. It provides all the suitable conditions for them to develop and flourish. To make our environment free of these bacteria, certain preventive measures should be taken

CONCLUSIONS

In the current study, all the collected samples of the meat's wash, were found to be contaminated with human pathogenic bacteria, samples of meat were collected from different localities of Lahore, Pakistan. Very effective techniques were used for the screening and identification of bacterial colonies. Different selective and differential media were used for the isolation and identification of bacterial colonies. It was concluded that exposed surfaces of meats contain pathogenic bacteria which are lethal to human life and they may cause harm or immediate death. So, there is a need for the contamination free environment to avoid the spread of pathogenic bacteria that causes very serious diseases in humans. This study will be helpful for the establishment of detection methods for pathogenic bacteria and creating awareness about contamination of pathogenic bacteria on raw meat.

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