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Antimicrobial Susceptibility Pattern Of *Staphylococcus aureus*: A Review

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Abstract

The study conducted to isolate *Staphylococcus aureus* and characterize from dairy product, to determine antimicrobial susceptibility for the isolates, and to identify hygienic practice condition considering dairy farms along with hygienic practice status associated with pathogens. Dairy breeds found in semi intensive production system, milk shop, and local market will be studied. The environment to be considered in the study includes faces from cattle house floor, milk container and equipment, milkier handle. Milk and milk product Samples will be collected from dairy farm and environment to isolate and detect *Staphylococcus aureus* and then test for antimicrobial susceptibility. Hygienic practice, sanitation and awareness status in relation to pathogens will also studied using structured questioner. The finding will have greater positive impact for the production and improvement of dairy production via preventing Zoonosis thereby improve public health.

Keywords: *Staphylococcus aureus*, Antimicrobial Resistance

Introduction

Staphylococcus aureus is gram positive cocci that occur in singles, short chains, tetrads and irregular grape like cluster. Only the strains that produce enterotoxin can cause food poisoning. The food handler with an active lesion or carriage later initiates infection. The *Staphylococcus* genus is subdivided into 32 species and subspecies. *Staphylococcus* Aureus produces Staphylococcal Enterotoxin (SE) and is responsible for almost all staphylococcal food poisoning. *Staphylococcus* Intermedius, a Staphylococcus species which is commonly associated with dogs and other animals, can also produce SE and has been rarely associated with staphylococcal food poisoning. The growth and survival of *S. aureus* is dependent on a number of environmental factors such as temperature, water activity (aw), and pH, the presence of oxygen and composition of the food. These physical growth parameters vary for different *S. aureus* strains (Stewart 2003). The temperature range for growth of *S. aureus* is 7–48°C, with an optimum of 37°C. *S. aureus* is resistant to freezing and survives well in food stored below -20°C; however, viability is reduced at temperatures of -10 to 0°C.*S. aureus* is readily killed during pasteurisation or cooking. Growth of S. aureus occurs over the pH range of 4.0–10.0, with an optimum of 6–7 (ICMSF 1996; Stewart 2003). *S. aureusis* uniquely resistant to adverse conditions such as low aw, high salt content and

osmotic stress. In response to low aw, several compounds accumulate in the bacterial cell, which lowers the intracellular awto match the external aw (Montville and Matthews 2008). As such, most *S. aureusstrains* can grow over an awrange of 0.83 to >0.99 (FDA 2012). S. aureusis a poor competitor, but its ability to grow under osmotic and pH stress means that it is capable of thriving in a wide variety of foods, including cured meats that do not support the growth of other foodborne pathogens (Montville and Matthews 2008). *S. aureus* is a facultative anaerobe so can grow under both aerobic and anaerobic conditions. However, growth occurs at a much slower rate under anaerobic conditions (Stewart 2003). For a non sporing mesophilic bacterium, *S. aureus* has a relatively high heat resistance (Stewart 2003). The observed average decimal reduction value (D-value, the value at which the initial concentration of bacterial cells would be reduced by 1 log10unit) was 4.8–6.6 min at 60°C when heated in broth (Kennedy *et al*. 2005). The bacteria has a higher heat resistance when it is encapsulated in oil, with a D-value at 60°C of 20.5 min for *S. aureus* in fish and oil (Gaze 1985). An extremely heat resistant strain of *S. aureus* (D-value at 60°C of >15 min in broth) has been recovered from a foodborne outbreak in India (Nema*et al*. 2007). 2 several chemical preservatives, including sorbates and benzoates, inhibit the growth of *S. aureus*. The effectiveness of these preservatives increases as the pH is reduced. Methyl and propyl parabens are also effective (Davidson and Taylor 2007).

The Staphylococcus species are host adapted with about one-half of the known species inhabiting humans solely and other animals. The largest numbers tent to be found near opening of the body surface such as the anterior nares, inguinal and perineal areas where in moist habitats, numbers per square centimeter may reach 1000-1000000 and in dry habitats 10-1000. The two most important sources to foods are nasal carriers and individuals whose hands and arms are inflicted with boils and carbuncles, which are permitted to handle foods (Quinn *et al*., 2001).

Insight towards isolation and identification of the possible sources of *Staphylococcus aureus* for effective control and prevention of the disease, as well as prevention of antimicrobial resistant of the bacteria and in addition to maintain hygienic practice for dairy production.

The objectives of this study are:-

- To isolate *Staphylococcus aureus* in different dairy farms.
- To assess hygienic practice and awareness status in dairy farms associated with pathogens.

Virulence factors and Pathogenesis

Staphylococcus aureus strains can express a wide array of potential virulence factors including surface proteins that promote adherence to damaged tissue bind proteins in blood to help evade antibody-mediated immune responses, and promote iron uptake. The organism also expresses a number of membrane-damaging toxins and super antigen toxins that can cause tissue damage and the symptoms of septic shock, respectively. There is a growing realization that S. aureus has multiple mechanisms for evading both innate immunity mediated by polymorphonuclear leukocytes and induced immunity mediated by both B and T cells (Bohach and Foster, 1998, Goodyear and Silverman, 2003).Some virulence factors are expressed by genes that are located on mobile genetic elements called pathogenicity islands (e.g., toxic shock syndrome toxin–1 and some enterotoxins; ref. 15) or lysogenic bacteriophages (e.g., Panton-Valentine leucocidin [PVL]) and factors associated with suppressing innate immunity such as the chemotaxis inhibitory protein and staphylokinase which are integrated in the bacterial chromosome (de Haas, 2004).

If food is stored for some times in room temperature the organism may in the food and can produce toxin. The bacteria produce enterotoxin while multiplying in food. *S. aureus* is known to produce six serologically different types of enterotoxins (A, B, C1, C2, D and E) that differ in

toxicity. Most food poisoning is caused by enterotoxin 'A' followed by type D. These enterotoxins are heat stable, with type B being most heat resistant. Enterotoxin stimulates Central Nervous Systems (CNS) vomiting center and inhibit water and sodium absorption in the small intestine. Staphylococcal enterotoxins, along with the toxic syndrome toxin and others, are classed as bacterial super antigens relative to in vivo antigen recognition in contrast to conventional antigens (Fedtke *et al*., 2004).

Symptoms

Food poisoning by *S. aureus* is characterized by a short incubation period typically 2-4 hours. The onset is sudden and is characterized by vomiting and diarrhea but no fever. The illness lasts less than 12 hours. In severe cases dehydration, masked pallor and collapse may require treatment (intravenously) infusion. The short incubation periods are the characteristics of intoxication where illness is the results of ingestion of the preformed toxin in the food (Adams and Moss 2008).

Detection

The presence of a large number of *S. aureus* organisms in a food indicates poor handling or sanitation. The dilution is placed on baird parker agar or mannitol salt agar. The enterotoxin can be detected and identified by gel diffusion (Radostits *et al*., 2007).

Antimicrobial Resistance and *Staphylococcus aureus*

The excessive use of antibiotics has led to the emergence of multiple drug resistant *S. aureus* strains. Infections caused by methicillin- or oxacillin-resistant *S. aureus* (MRSA) are mainly nosocomial and are increasingly reported from many countries worldwide. As MRSA strains are frequently resistant to many different classes of antimicrobial drugs, second- and third-line antimicrobial resistance is a growing concern. Surveillance of MRSA provides relevant information on the extent of the MRSA epidemic, identifies priorities for infection control and the need for adjustments in antimicrobial drug policy, and guides intervention programs (WHO, 2001). In most cases, resistance to antibiotics is coded for by genes carried on plasmids, accounting for the rapid spread of resistant bacteria. Soon after the in-troduction of methicillin, described the emergence of methicillin resistant *S. aureus* (MRSA), which have since spread worldwide as nosocomial pathogens.

Economic Importance of *Staphylococcus aureus*

Healthcare- associated MRSA is a major cause of nosocomial infections worldwide, with significant attributable morbidity and mortality in addition to pronounced healthcare cost. Although methicillin-resistant *Staphylococcus aureus* (MRSA) is an increasingly common pathogen, the independent contribution of methicillin resistance to the outcomes for patients with *S. aureus* infection is unclear because patients who develop MRSA infections are typically older and sicker than are patients who develop methicillin susceptible *S. aureus* (MSSA) infection. Surgical site infection (SSI) complicates 2%–5% of all Received surgeries in the United States, resulting in a total of 300,000–500,000 infections each year. SSIs are associated with increased morbidity rates, mortality rates, and costs, and they are responsible for additional nannual hospital charges of~$1.6 billion in the United States alone, S. aureusis a virulent pathogen and the most common cause of SS. Methicillin re-sistance further complicates therapy for S. aureus SSI. The prevalence of MRSA has increased dramatically since it was first described in the 1960s (NNIS, 2001).

Control and prevention:

Food born bacterial illness by bacteria are most commonly prevented and controlled by proper cooking and preparing of food as well as storing. For examples adequate refrigeration of food, improve personal hygiene, adequate cooking and heating processing. The control method or measures also includes; a) education of those who prepare the food at home and other food handlers, so that they have to take proper personal

measures; b) prohibiting individuals with absences or other skin lesions from handling food; c) placing of food in cold place at 4 degree centigrade or lower of all food in order to prevent bacterial multiplication and the formation of toxin. Foods must be kept at room temperature for as little time as possible (W H O, 2008)

The oxidase test

The oxidase test is a biochemical reaction that to assays for the presence of Cytochrome oxidase, an enzyme sometimes called indophenol oxidase. There are many method variations to the oxidase test. These include, but are not limited to, the filter paper test, filter paper spot test, direct plate method, and test tube method. Filter Paper Test Method: Soak a small piece of filter paper in 1% Kovács oxidase reagent/Tetrametyl-p-phenylenediamine di-hydrochloride (1% water solution) and let dry. Then using a loop and pick well isolated colony from a fresh (18 to 24 hour culture) bacterial plate and rub onto treated filter paper. Microorganisms are oxidase positive if the color changes to dark purple within 5 to 10 seconds. Microorganisms are delayed oxidase positive when the color changes to purple within 60 to 90 seconds. Microorganisms are oxidase negative if the color does not change or it takes longer than 2 minutes which indicate a positive reaction for *Escherichia coli.*

Indole test

The indole test screens for the ability of an organism to degrade the amino acid tryptophan and produce indole. It is used as part of the IMViC (indole, MR-Vp Citrate) procedures, a battery of tests designed to distinguish among members of the family Enterobacteriaceae. /*E. coli*, *Vibrio cholerae*. Inoculate the tube of tryptone broth with a small amount of a pure culture then incubate at 37°C for 24 to 48 hours. Add 5 drops of Kovác's reagent directly to the tube to test for indole production. Formation of a pink to red color (“cherry-red ring”) in the reagent layer on top of the medium within seconds of adding the reagent indicated a positive indole test for *E. coli.* If a culture is indole negative, the reagent layer will remain yellow or be slightly cloudy.

Triple Sugar Iron (TSI) Agar

Triple Sugar Iron Agar Medium is recommended for identification of gram-negative enteric bacilli on the basis of dextrose, lactose and sucrose fermentation and hydrogen sulphide production in accordance with Indian Pharmacopoeia. Suspend 64.42 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml purified/ distilled water. Heat to boiling and dissolve the medium completely. Mix well and distribute into test tubes and Sterilize by maintaining at 10lbs pressure (115°C) for 30 minutes or as per validated cycle. Allow the medium to set in sloped form with a butt about 2.5cm long. Directions specified are as per the concurrent edition of pharmacopoeia in force. Specified expiry period corresponds to this. Peptone, yeast extract and beef extract provide nitrogenous compounds, sulphur, trace elements and vitamin B complex etc. Sodium chloride maintains osmotic equilibrium. Lactose, sucrose and dextrose monohydrate are the fermentable carbohydrates. Sodium thiosulphate and ferric or ferrous ions make H2S indicator system. Sodium thiosulphate is also an inactivator of halogen and can minimize its toxicity in the testing sample, if any during microbial limit tests. Phenol red is the pH indicator. Bacteria that ferment lactose or sucrose (or both) produce large amounts of acid enables no reversion of pH in that region and thus bacteria exhibit an acid slant (respiration) and acid butt region (fermentation). Gas production (CO2) is detected by the presence of cracks or bubbles in the medium and in H2S no blackening of medium or negative which will be indicate positive result for Enterobacteriaceae such as *Escherichia coli* strains and *Klebsiella* species then further they will be differentiate by other different biochemical tests such as motility test indole test, Urease test, simmon citrate, Methyl Red and others.

The reactions can be summarized as follows:Acid slant / acid butt - dextrose and sucrose fermented or dextrose and lactose fermented or all the three sugars, dextrose, lactose and sucrose fermented. Bubbles or cracks present - gas production, Black precipitate present - H2S gas production.

Citrate Test

The citrate test screens a bacterial isolate for the ability to utilize citrate as its carbon and energy source. A positive diagnostic test rests on the generation of alkaline by-products of citrate metabolism. The subsequent increase in the pH of the medium is demonstrated by the color change of a pH indicator. The citrate test is often part of a battery of tests used to identify gram-negative pathogens and environmental isolates.

Use a fresh (16- to 18-hour) pure culture as an inoculation source. A single isolated colony and will be Pick and lightly streak the surface of the slant. A needle is the preferred sampling tool in order to limit the amount of cell material transferred to the agar slant. Avoid using liquid cultures as the inoculum source. Citrate utilization requires oxygen and thus screw caps, if used, should be placed loosely on the tube. Incubate at 35oC (+/- 2oC) for 18 to 48 hours. If trace or no growth will be visible, no color change will occur; the medium will remain the deep forest green color of the un-inoculated agar which indicates a positive reaction for *Escherichia coli*.

Urease test

The urease test identifies those organisms that are capable of hydrolyzing urea to produce ammonia and carbon dioxide. It is primarily used to distinguish urease-positive bacteria from other Enterobacteriaceae. A heavy inoculum from an 18- to 24-hour pure culture will be used to streak the entire slant surface. Do not stab the butt as it will serve as a color control. Incubate tubes with loosened caps at 35 o C. Observe the slant for a color change at 6 hours, 24 hours, and every day for up to 6 days. Urease production is indicated by a bright pink (fuchsia) color on the slant that may extend into the butt. Delayed-positive organisms (e.g.,Klebsiellaor Enterobacter) will typically produce a weak positive reaction on the slant after6 hours, but the reaction will intensify and spread to the butt on prolonged incubation (up to 6 days). A negative urease test is indicated by no color change (yellowish-orange) or a yellow color change in the medium which indicate positive reaction for *Escherichia coli*.

Conclusion and Recommendations

Staphylococcus aureus are the main microbiological hazards among the most pathogenic bacteria that linked to raw milk. The study conducted to isolate and characterize *Staphylococcus aureus* from dairy product, to determine antimicrobial susceptibility for the isolates, and to identify hygienic practice condition considering dairy farms along with hygienic practice status associated with pathogens. Dairy breeds found in semi intensive production system, milk shop, and local market will be studied.

The following recommendations forwarded:-

- The environment should be considered in the study includes faeces from cattle house floor, milk container and equipment and milkier handle.
- Milk and milk product Samples should be collected from dairy farm and environment to isolate and detect *Staphylococcus aureus* and then test for antimicrobial susceptibility.

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