С.Сейфуллин атындағы Қазақ агротехникалық университетінің **Ғылым жаршысы (пәнаралық)** = **Вестник науки** Казахского агротехнического университета им. С.Сейфуллина (**междисциплинарный**). - 2018. - №2 (97). - P.140-145

ASSESSMENT OF MICROBIOLOGICAL QUALITY OF RETAIL HORSEMEAT PRODUCTS

Kairova M.G.

Annotation

This research was done by using traditional microbiological and biochemical methods, two samples of horsemeat products were tested to assess their microbiological safety. Number of colony-forming units in the original meat samples were 1.1 - 7.8 x 10^5 CFUs per 1 gram. There is about same level as it is determined for frozen blocks of meat according to Technical Regulations of Custom Union. It concluded that all studied bacterial isolates from meat samples are not coagulase positive pathogenic S. aureus according to resulted tests such as coagulase, catalase, oxidase and hemolysis reactions and others.

Key words: bacterial isolates, horsemeat, staphylococci, coagulase-negative microorganisms, microbiological safety

Introduction

Natural populations of cocci are mainly associated with warm-blooded animals and birds. Some of them can be isolated from variety of animal products and environmental sources and regarded as commensal staphylococci and streptococci. Other cocci species under appropriate conditions can cause humans and/or animals infections [1].

Staphylococci have become remarkably wide spread in the foods such as chicken products [2, 3], meat, milk and milk-derived products [4, 5]. Staphylococcus aureus is one of five pathogens that common causing foodborne human illness throughout the world [6]. In Europe 53% of the cases of food-outbreaks reported in 1969-1990 were due to staphylococcal food poisoning [2]. This type of food poisoning is still reported and S. aureus causing about 241000 illnesses annually in US [6]. The etiological agent of staphylococcal food poisoning is a variety of extracellular toxins (enterotoxins), many of which are heat-resistant in foods. According to recent studies most strains of S.

aureus produce a group of 15-21 enterotoxins and among them Staphylococcal enterotoxin A (SEA) causes more than 50% of food poisoning [7]. Genetic and physiological variability of S. aureus strains allow to a commensal bacterium become a powerful pathogen [8]. Before it was known that only coagulase-positive S. aureus, S. intermedius, S. delphini, and S. schleiferi subsp. coagulans and the coagulase variable S. hyicus are opportunistic pathogens of human and animals. However, coagulase-negative (CoNS) S. epidermidis strains and other species are capable of causing bacteremia and can produce enterotoxins [9].

The present study was undertaken to research a bacterial population of cocci in retail horsemeat products that pose a potential risk for food safety.

Materials and methods.

Two refrigerated retail horsemeat products were obtained from the local market in Akmola and North Kazakhstan provinces. The meat samples were aseptically collected, and each meat probe was placed in a separate, sterile plastic bag. The samples were brought under refrigeration to the laboratory and analyzed.

The mixed sample of meat diluted in a series of tubes containing sterilized physiological saline and then suitable dilutions were spread onto the surface of agar plates in 2 or 3 repetitions. The number of colony-forming units (CFUs) in the original sample determined by the spread plate technique. Totally nine bacterial strains were isolated from two horsemeat samples. Isolation was carried out on Nutrient agar (Titan, India). The bacteria were maintained on nutrient agar slants and stored at 4^0C.

Catalase test. The test was done by slide method and using a sterile 24-hour bacterial culture and a fresh 3% hydrogen peroxide.

Oxydase Test. Strains were cultivated at 30^0C on Nutrient agar. After 20 h of incubation, one loop of bacteria was spread on an oxidase disk (Fluka, #70439).

Growth in a Glucose-Contained Medium. One loopful of a 24-h culture in Nutrient agar was transferred to a tube of Gissa medium. The inoculated tubes were incubated at 35^0 C for 48-72 h under anaerobic condition.

Morphological characterization. Staphylococcus agar (Fluka, #70193) was used for phenotypic characterization of cocci isolates based on type of growth. On this medium bacterial colonies were also tested on mannitol fermentation based on color changing of 0.04% bromtymol blue as a possible indicator.

Coagulase Test. The coagulase test is used to distinguish between pathogenic and nonpathogenic members of the genus Staphylococcus. All pathogenic strains of S. aureus are coagulase positive whereas the nonpathogenic species (S. epidermidis) are coagulase negative.

Overnight cultures on Nutrient broth and 1 ml of 1 in 10 diluted rabbit plasma were used for a coagulase test. The formation of a clot

was examined at half hourly intervals for a period of four hours, and 24 h. Any gelling of the plasma was considered a positive reaction.

Carbohydrate Dissimilation. The production of acid from mannitol under aerobic conditions was tested on Gissa medium (Biotechnovation, Russia). Incubation of bacterial culture was carried out at 35^0C for 3 d, with daily readings.

Results and discussion

The number of colony-forming units (CFUs) in the original meat samples were determined by using the spread plate technique. After the incubation time, the well-isolated colonies developed on the agar medium are counted in some plates that appear to have between 30 – 300 colonies. Therefore, in the average 112 ±23.8 CFUs were obtained on the agar plates that inoculated by suspension containing 10^{-2} dilution of meat sample from the North Kazakhstan. In contract that, the agar plates are having 78 ±5.6 CFUs represents 10^{-3} dilution of meat suspension from the Akmola province. In totally plate count technique are allowed to determine that original meat products contain 1.1×10^5 and 7.8×10^5 CFUs per 1 gram in samples from the North Kazakhstan and Akmola provinces.

Nine isolated bacterial colonies were randomly picked up to study their purity, morphological and physiological properties. All of studied isolates have circular shaped, convex, opaque, white or beige colonies, except clearly yellow-pigmented 1KG and orange colored of 1KS cultures. Pigmentation of staphylococcal culture can act as virulence factor and protects from reactive form of oxygen. For this purpose, studied bacterial isolates were cultivated on standard Staphylococcus agar medium to determine a pigment characteristic of colonies.

In during 24 hours cultivation of nine isolates, only five of them 1KB, 1KP, 2KB1, 2KB2 and 2KY1 showed abundant and rapid growth on the streaked selective agar plates. As shown in the figure 1, four cultures 2KB1, 2KB2, 2KR1 and 2KY1 isolated from the Akmola meat sample had white or slightly yellow colonies on this selective media. However, among these cultures, 2KR1 and 2KY1 strains can develop yellow pigmentation late in incubation as well as 1KB isolate.

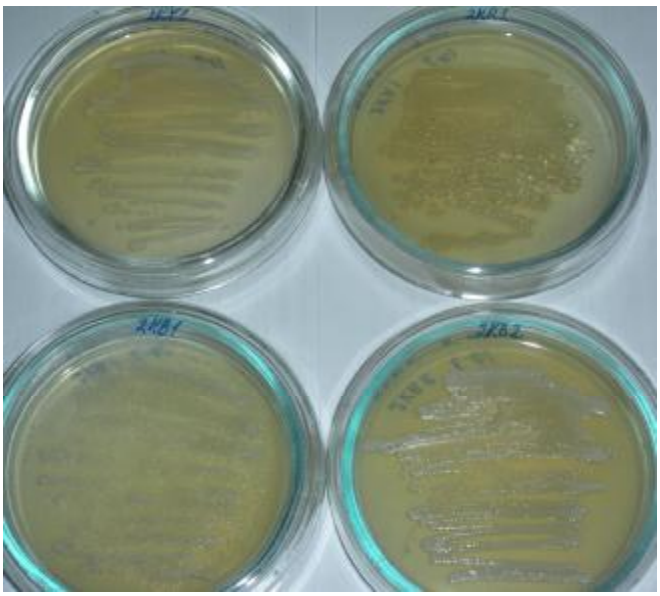


Figure 1 - Cultivation of bacterial isolates from the Akmola province meat sample

Purified cultures were tested for Gram staining. Isolates 1KY, 1KB, 1KP, 1KG, 1KS from the North Kazakhstan meat sample, and 2KB1, 2KB2, 2KR1 and 2KY1 from the Akmola province sample were Gram-positive and non-motile bacteria with spherical or ovoid cells. In particularly, cells of isolates 1KB, 1KP, 2KB1 and 2KB2 occurring in pairs, in short chains and also can form irregular grape-like clusters as shown in the figure 2 for 2KB2 isolate. This type of cell arrangement is characteristic for the genus Staphylococcus [1].

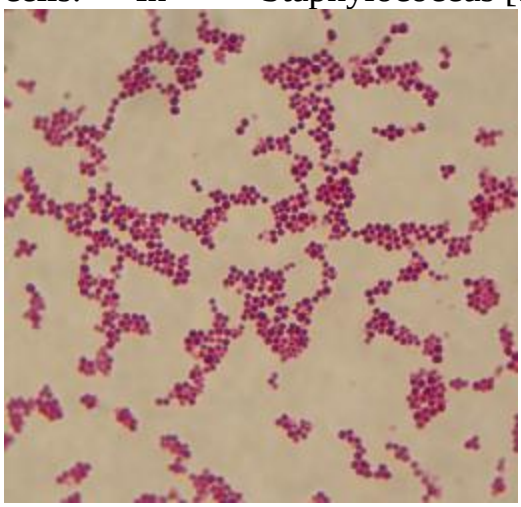


Figure 2 - Gram-stained cells of 2KB2 isolate from the Akmola province meat sample

All bacterial isolates were cultivated aerobically at 35^0C on the differential Gissa medium containing carbohydrate mannitol and the indicator phenol red to distinguish mannitol-fermenting pathogenic staphylococci. Resulting this differential medium had no color changed indicating that isolated coccal forms of bacteria were not able to ferment a mannitol as shown for three meat cultures in figure 3.

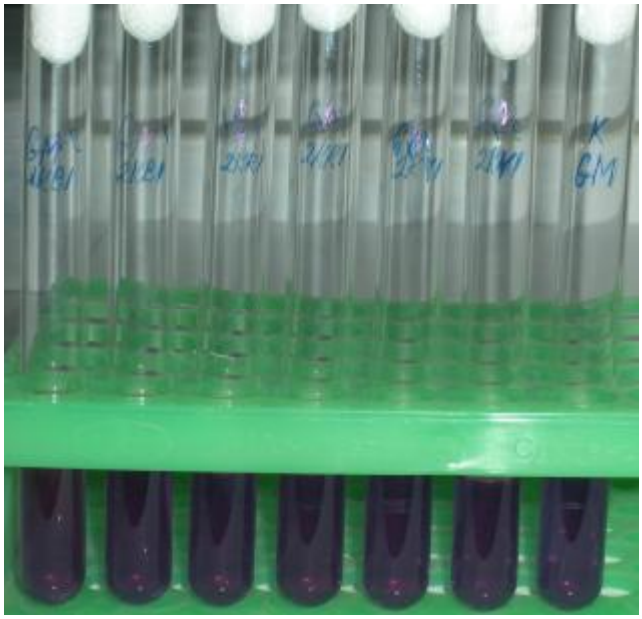


Figure 3 - Cultivation of bacterial isolates from the Akmola province meat sample on mannitol containing medium

The most staphylococcal species are able to ferment a glucose under anaerobic conditions with the production of acid. It is also used as genus specific characteristics to differentiate staphylococci and micrococci. In the figure 4 shown an ability of isolates from the North Kazakhstan meat sample to utilize a glucose in anaerobic condition. Only 1KY, 1KP, 1KB, 2KR1 strains were capable more efficiently to turn a color of differential glucose contained medium to yellow that indicates they can utilize this carbohydrate.

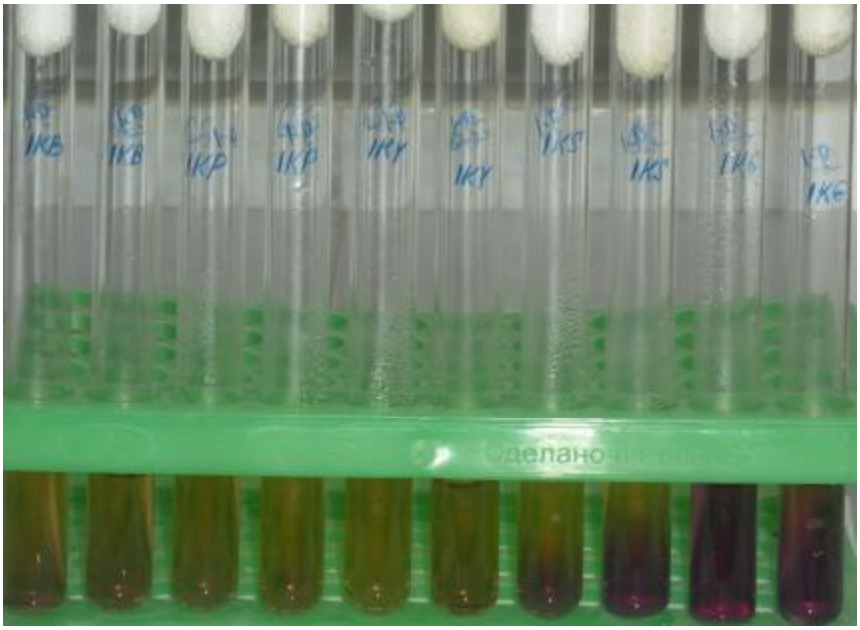
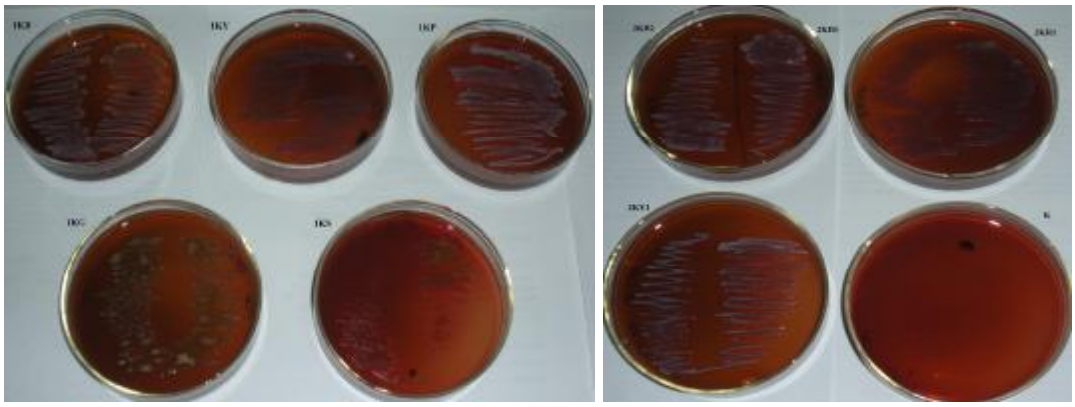


Figure 4 - Cultivation of bacterial isolates the North Kazakhstan meat sample on glucose containing medium

On blood agar plates, bacterial colonies are not surrounded by clear beta-hemolysis zones as it is presented in figure 5 for all nine cultures. This result are indicating that researched strains do not belong to pathogenic bacteria [10].



A B

Figure 5 - Cultivation of bacterial isolates from meat sample on Blood agar medium: A – isolates from the North Kazakhstan meat sample and B - isolates from the Akmola meat sample

None of nine bacterial isolates showed clot formation within a period of four hours, and 24 h at 37^0C. In the resulted test, studied bacterial cocci were coagulase-negative microorganisms. Bacterial strains were tested for oxidase and catalase activities by using related protocols for an oxidase disk (Fluka, #70439) and 3% hydrogen peroxide. All studied nine isolates showed negative reaction for oxidase and were catalase-positive.

By using traditional microbiological and biochemical methods, two samples of horsemeat products were tested to assess their microbiological safety. Enumeration of amount of mesophylic aerobes and facultative anaerobic microorganisms in the retail horsemeat was done by the spread plate technique on Nutrient agar.

Generally, number of colony-forming units (CFUs) in the original meat samples were 1.1 - 7.8 x 10^5 CFUs per 1 gram. There is about same level as it is determined for frozen blocks of meat according to Technical Regulations of Custom Union “About Safety of Food Products” (2011). In this study, any gram-positive rod-shaped bacteria and gram-negative coliforms were not detected that indicating lack of soil and fecal contaminations in the meat samples. Presumptive nine staphylococcal isolates from meat products were examined to determine their pathogenicity [8]. Used wide range of biochemical tests such as coagulase, catalase, oxidase and hemolysis reactions are allowing to conclude that all bacterial isolates not belonging to coagulase positive pathogenic S. aureus.

There are 1KY, 1KB, 1KP strains isolated from the North Kazakhstan meat, and 2KB1, 2KB2, 2KR1 and may be 2KY1 strains

isolated from the Akmola meat sample can be determined as coagulase negative staphylococci. Non-one bacterial cultures were able aerobically to utilize a mannitol and among these isolates only 1KY, 1KP, 1KB, 2KR1 strains were able to ferment glucose in anaerobic condition. Known that most strains of S. epidermidis not mannitol fermenting and some of them could not anaerobically utilize glucose substrate. In following our researches using modern molecular techniques for staphylococci identification are more preferable because of coagulase-negative S. epidermidis and S. haemolyticus strains are causing agents of bacteremia and can produce enterotoxins [11].

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ОЦЕНКА МИКРОБИОЛОГИЧЕСКОГО КАЧЕСТВА РОЗНИЧНОГО МЯСА КОНИНЫ

Каирова М.Ж.

Резюме

С использованием традиционных микробиологических и биохимических методов проведена оценка микробиологической безопасности двух образцов конского мяса. Количество колониеобразующих единиц в исследуемых образцах мяса составило 1,1 – 7,8 x10^5 КОЕ на грамм. Согласно Техническому регламенту Таможенного союза это соответствует допустимому уровню для замороженных блоков мяса. На основе тестов на коагулазу, каталазу, оксидазу, гемолитическую активность и другие показано, что изоляты выделенные из мяса не являются патогенными коагулаза-положительными стафилококками.

Ключевые слова: бактериальные изоляты, конское мясо, стафилококки, коагулаза-негативные микроорганизмы, микробиологическая безопасность

САТЫЛЫМДЫ ЖЫЛҚЫ ЕТ ӨНІМДЕРІНІҢ МИКРОБИОЛОГИЯЛЫҚ САПАСЫҢ БАҒАЛАУ

Каирова М.Ж.

Микробиологиялық және биохимиялық әдістері көмегімен екі жылқы ет сынамаларының микробиологиялық қауыпсыздығы бағаланып зерттелді. Осы 1 грамм жылқы ет сынамаларында микроағзалардың саны 1,1 - 7,8 x10^5 КОЕ аралығында болды. Бұл сан Кеден Одағының Техникалық регламентімен мұздатылған ет блоктарына қойылған шектеу деңгейне сәйкес боп табылады. Коагулаза, каталаза, оксидаза, гемолитикалық активтігін және тағы басқа зерттеулер барысында еттен бөліп алынған бактерия изоляттарың патогенды коагулаза-позитивті стафилакокктарға жатқызуға болмайды.

Түйін сөздері: бактериалды изоляттар, жылқы еті, стафилококктар, коагулаза-негативті микроорганизмдер, микробиологиялық қауыпсыздық