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Original Research Paper

THE SOURCE OF CONTAMINATION OF GROUND MEAT FOR PRODUCTION OF MEAT PRODUCTS WITH BACTERIA *STAPHYLOCOCCUS AUREUS*

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Summary: In a plant for beef-slaughtering we established the sources of contamination of beef carcasses and ground meat, made from meat of beef carcasses and which are the most important places of contamination in the production process. We took altogether 250 smears from the surface of beef carcasses. The specimens were taken on five different areas on beef carcasses. The bacteria *S. aureus* was isolated on the thorax in 78 % (39/50) of the specimens, 62 % (31/50) on the front legs, 58 % (29/50) on the abdomen wall, 14 % (7/50) on the thigh and 10 % (5/50) on the neck. The established contaminations of workers' hands was 50 % of specimens before the beginning of the work and 58.33 % of specimens taken after the handling of five slaughtered carcasses and are an important source of contamination of meat.
For differentiation of separate strains of bacteria *S. aureus* we used the RAPD-PCR method and four different oligo-nucleotide primers OPJ5, OPJ6, E7 and E8. With genotypification of oligo-nucleotide primers OPJ%, E8 we proved the correlation between ground meat, hands, equipment and beef carcass.
Because the surfaces of beef carcasses are contaminated with the same type of bacteria
S. aureus as the hands of workers, establishing contamination of the surface of beef carcasses with bacteria *S. aureus* on the slaughtering line can be the indicator hygiene of work among workers on the slaughtering line.
The major source of contamination of ground meat are the hands of workers (contamination of workers' hands with bacteria *S. aureus* was 58.33 % of the specimen taken after the handling of five beef carcasses) and the contaminated surface of beef carcass (44.4 % of smears of surface of beef carcasses was contaminated with bacteria *S. aureus*).
The contaminated surface of beef carcasses is crucial for contamination of ground meat as the meat goes to grinding.

Key words: food inspection; food contamination; meat-microbiology; *Staphylococcus aureus*-isolation and purification

Introduction

Staphylococci are ubiquitous in nature. *S.aureus* is the most important species in the group as some strains are capable of causing human foodborne intoxication. The primary reservoir is on the skin and mucous membranes of mammals and birds. *S. aureus* is frequently isolated from ground meat. Enterotoxigenic strains of *S. aureus* in ground meat can grow to sufficient level to allow a toxic dose of enterotoxin to be produced prior to consumption. Enterotoxin is thermo-stable and is not destroyed with heat treatment. Because of the enterotoxin thermo-stability, level of contamination of meat is to be kept as low as possible during the production process. The initial contamination of meat occurs during slaughtering. Hygiene deficiencies cannot be compensated for even by the most rigorous hygiene measures during later production process. Microbiological hygiene measures in meat production aim at protecting the consumer against pathogenic agents. To prevent contamination of meat with *S. aureus*, sources of *S. aureus* must be determined and well known.

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Molecular test, Random Amplified Polymorphic DNA (RAPD-PCR), is a useful method for differentiation of strains of *S. aureus*. Several authors reported that a strain of S. aureus isolated from cows with mastitis can be differentiated using a PCR method with random primers. This method is preferable over biotyping, as it is a much more specific method (1). Molecular methods such as PCR-based DNA fingerprinting may be useful for epidemiological purposes (2).

Material and methods

Collection of samples

Swabs: Swabs were taken with sterile cotton sticks in mannitol salt broth (Mannitol salt broth-Biolife Italiana S.r.l.) from a surface of 25 cm^2. We limited the area with a sterile, paper model.

Swabs were taken from surface of equipment on the slaughter line and in the cutting room, from hands of workers on the slaughter line and in the cutting room and from the surface of carcasses on five different areas on the carcass; thigh, abdomen wall, thorax, front leg and neck.

Cough up air samples: Samples were taken directly on Baird –Parker broth during coughing of workers.

Ground meat samples: Samples were taken in a sterile plastic bag immediately after grinding.

Isolation and identification of S. aureus

Swabs were incubated in mannitol salt broth (Mannitol salt broth- Biolife Italiana S.r.l.) at a temperature of 37°C for 24 hours. 1g of ground meat is diluted in 9 ml of mannitol salt broth.

Mannitol salt broth was spread on Baird –Parker agar (Baird –Parker agar Staphylococcus Selective Agar base acc. to Baird –Parker) and incubated at 37°C for up to 48 hours. Colonies which exhibited typical morphology (grey-black shiny convex colonies, 1-1.5 mm in diameter with a narrow white entire margin surrounded by a zone of clearing, 2-5mm) were spread on blood agar

(Blood Agar Base N°2+5% blood) and incubated at 37°C for up to 48 hours. Colonies were tested for coagulase production using rabbit blood plasma (BBL ™ coagulase plasma, rabbit with EDTA). Additional phenotypic traits were used with a commercial kit (BBL CRYSTAL ™ GP Identification System, Gram-Positive ID Kit).

DNA extraction

DNA from bacterial cultures was extracted by the PROMEGA method. (Wizard® Genomic DNA Purification Kit) according to the manufacturer instructions. For destruction of the bacteria cell wall lisozim and lisostafin were used.

DNA amplification

Amplification was performed with RAPD-PCR. For differentiation of separate strains of bacteria *S. aureus* we used the RAPD-PCR method and four different oligo-nucleotide primers OPJ5, OPJ6, E7 and E8 (2).

Results

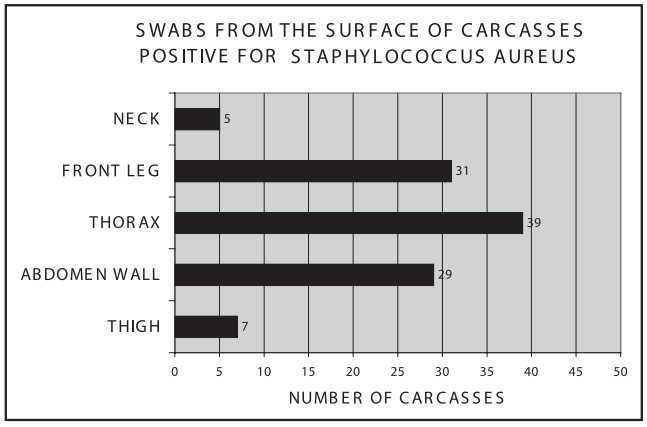
For establishing the hygiene status of the establishment 140 swabs were taken from surfaces of equipment. Established contamination (> 100 micro-organisms/cm^2) was 6.42% (9/140) of specimens. For establishing contamination before the beginning of work with *S. aureus* on surfaces of equipment we took 40 specimens. *S. aureus* was isolated from 5% (2/40) of specimens. In addition we took 41 specimens from equipment after the handling of five beef carcasses. *S. aureus* was isolated from 46.34% (19/41) of specimens.

For establishing contamination of workers hands, 24 swabs were taken before the beginning of the work and 24 swabs after handling five beef carcasses. S. aureus was isolated from 50% (12/24) of specimens and 58.33% (14/24) of specimens, respectively. From 10 specimens of coughing up air of workers, S. aureus was recovered from 10% (1/10) of specimens.

From the surface of beef carcasses samples were taken from five different areas on the carcass. *S. aureus* was isolated from 78% (39/50) of specimens taken from the thorax area, 62% (31/50) of specimens taken on the front leg, 58% (29/50) of specimens taken on the abdomen wall, 14% (7/50) of specimens taken on the thigh and 10% (5/50) of specimens taken from the area of the neck. Altogether, S. aureus was isolated from 44.4% (111/250) of specimens taken from surfaces of beef carcasses.

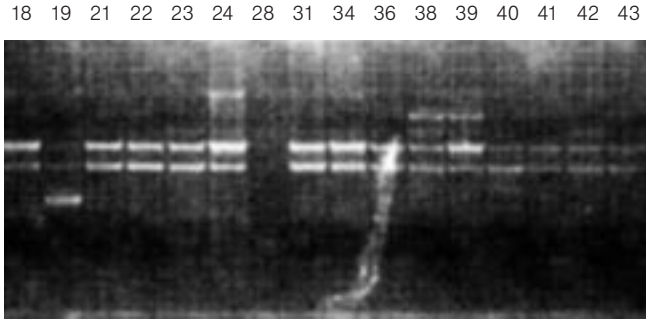
From ground meat specimens *S. aureus* was recovered from 62.5% (10/16) specimens. Results of genotyping of S. aureus, isolated from specimens taken in slaughterhouse by the RAPD-PCR method.

Table 1: Swabs taken from the surface of carcasses positive for *S. aureus*



With oligo-nucleotide primer OPJ 5 we got 4 different profiles of DNA, designated as A, B, D, C profile. Profile A: We got the same profiles of DNA of *S. aureus* isolated from workers hands before the beginning of work, knife and surface of carcasses. Profile B: We got the same profile from specimens taken from humans, bovine and surface of beef carcasses. Profile C: We got the same profile from specimens taken from hands of workers before the beginning of work, from hands of workers taken after handling five beef carcasses, specimens taken from the surface of carcasses and specimens of bovine origin. Profile D: We got the same profile from specimens taken from hands of workers after handling five beef carcasses and specimens of bovine origin. With oligo-nucleotide primer OPJ 6 we did not obtain any results.With oligo-nucleotide primer E 7 we got different profile of DNA.

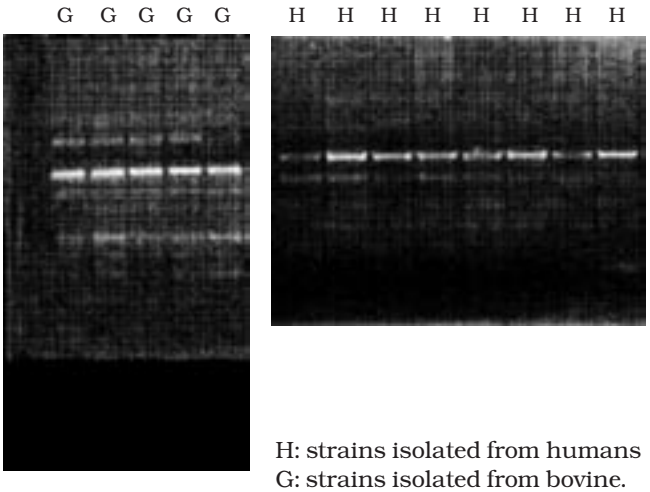
Figure 1: Results of genotyping of S. aureus, isolated from specimens taken in slaughterhouse with the RAPD –PCR method, with oligo-nucleotide primer E 7



38, 39: strains isolated from ground meat

The profile of DNA of the strain isolated from the hands of workers (sample 21, 22, 40) was the same as the profile of DNA of strains isolated from the surface of beef carcasses (sample 23, 31, 34) The profile of DNA of strains isolated from ground meat was different from the strains isolated from humans and bovine. We got a different profile of DNA isolated from humans and bovine.

Figure 2: Results of genotyping of *S. aureus*, isolated from specimens taken in slaughterhouse with the RAPD –PCR method, with oligo-nucleotide primer E 7.



H: strains isolated from humans
G: strains isolated from bovine.

Strains isolated from humans and bovine have a different profile of DNA.

With oligo-nucleotide primer E8 we got 2 different profiles of DNA. The profile of DNA of the strain isolated from the hands of workers was the same as the profile of DNA of strains isolated from the surface of beef carcasses. *S. aureus* isolated from the knife have the same profile of DNA as strains isolated from humans and from ground meat. Strains isolated from ground meat have the same profile as strains isolated from humans and bovine.

Discussion

S. aureus is frequently isolated from food samples. The high incidence of staphylococci on beef carcasses is of concern, since they can act as a source of contamination to other foods.

S. aureus can present a risk to other foods especially those that are not subject to a bactericidal process.

The objective of our study was to establish the correlation between sources of bacteria *S. aureus* and ground meat and determine weather the strains

of *S. aureus* are of human or animal origin.

Equipment in the slaughterhouse was contaminated before the beginning of work. Although this contamination may to a certain extant be difficult to avoid, the level of contamination can be substantially increased or decreased by poor or good slaughter procedures, respectively. Contamination of muscle tissue during the slaughter process may occur as a result of direct or indirect contact with e.g. faeces, skin, contaminated tools and equipment, personnel and clothing. In this study 5 % of samples from equipment were contaminated before the beginning of work, 46.34 % of samples were contaminated after handling five carcasses. During the process of meat production the contamination was raised. According to these results, the probability of contamination of carcasses had been raised during the production process.

Contamination of workers hands was high, 50 % (12/24) of samples, already before the beginning the work, and it was raised after handling five carcasses to 58.33 % (14/24) of samples. The hands of workers are an important primary source of contamination of products with *S. aureus* during meat processing (3). *S. aureus* is present on the skin of hands, and hands are also contaminated through contact with surfaces that are rich in bacteria, such as the skin of slaughtered animals, intestinal contents, abscesses. Especially, hands can be a source of contamination if infected cuts and sores are present. During evisceration, and to a lesser extent trimming, workers have considerable contact with - and have to handle - the carcasses around the brisket and the flank. The hands of evisceration workers and trimming staff at the slaughterhouse were shown to be heavily contaminated with coagulase positive staphylococci (CPS), while the hands of non-meat workers from the same slaughterhouse were shown to be infrequently contaminated (4). Hands of evisceration workers were continually wet and were subjected to rough physical activities. Their hands had signs of skin damage and this poor skin condition could have led to increased colonisation by staphylococci, accounting for the high numbers present on hands at the commencement of work (4). Hand washing has a variable effect on the reduction of bacteria on hands, depending on the mechanical action and the duration, together with the type of soap or sanitisers used (5).

Contamination of the surface of carcasses was examined with 250 smears from the surface of meat on five different areas. The bacteria *S. aureus* was isolated on the thorax in 78% (39/50) of the specimens, 62% (31/50) on the front legs, 58% (29/50) on the abdomen wall, 14% (7/50) on the thigh and 10% (5/50) on the neck. Contamination was shown to be the highest on the thorax and front leg of carcasses. The area of the abdomen wall was still high, and areas of thigh and neck were contaminated to a lesser extent. Underlying tissue of the hide in a healthy animal is sterile. The initial contamination of meat occurs during slaughtering. The level of contamination is in correlation with working operations on the slaughter line. Working operations in the production of meat: hide removal, evisceration, splitting of carcasses, trimming and washing of surface, handling of carcasses all contribute to contamination of the meat. The level of contamination is apparently in correlation with working operations on the slaughter line and the time and temperature of holding the meat.

The process of hide removal results in some microbiological contamination of the underlying carcass tissue, and the subsequent extent of the contamination depends on the technique used and the level of contamination on the hide (6). Contamination with CPS was increased after evisceration and to a lesser extent after trimming (4).

Hygienic practice was found to be associated with the carcass contamination level, especially disinfection frequency. Designing slaughtering lines so as to make hygienic work possible is very important (7).

Chilling temperatures are not low enough to prevent growth of *S. aureus*. Production of enterotoxins is related to growth and it is unlikely that the growth of CPS during weekend chilling of beef carcasses poses an immediate health risk. A longer period of refrigeration would be required before enterotoxins were detected (4).

S. aureus was isolated also out of 10% (1/10) of specimens of cough up air of workers on the slaughtering line. Samples of cough up air suggests that the workers can contribute to contamination also in this way. The environment may also contribute to bacterial cross-contamination of carcasses because of the presence of CPS in air samples (4).

Associations between the microbiological contamination of air and carcasses with the movements of workers were found (7).

The presence of *S. aureus* in ground meat was found in 62.5% (10/16) of specimens. The presence of *S. aureus* in ground meat is not an immediate health risk. Storage of such contaminated ground

meat in an inadequate environment for a longer period of time could enhance the growth of bacteria and production of enterotoxins, especially in meat products that are not subject to a bactericidal process.

Animal biotypes were isolated from workers in the slaughterhouse. Humans could act as a reservoir for both human and animal biotypes (8).

For differentiation of separate strains of bacteria *S. aureus* we used the RAPD-PCR method. We proved a correlation between beef carcasses, hand equipment and ground meat, and we established differences in RAPD-PCR patterns between isolated strains of *S. aureus* from human and animal origin. In the slaughterhouse we isolated both types of strains from workers hands, equipment beef carcass and ground meat .

These findings support the results of other studies, that coagulase positive staphylococci isolated from workers in slaughterhouses have similar phage patterns to strains isolated from meat products (3).

According to the findings from our study the hands of workers are a source of contamination of the surface of beef carcasses with bacteria *S. aureus*. The surface of carcasses frequently is comes into contact with hands during operations on the slaughter line.

The contaminated surface of beef carcasses is crucial for contamination of ground meat as the meat goes to grinding. To prevent the contamination of carcasses it is essential that the incidence and counts of *S. aureus* be reduced. To achieve this, more care needs to be exercised in removing the hide, and viscera, and in carefully trimming the surface of carcasses prior to chilling. Washing of the carcass may have resulted in redistribution of the contamination (3). It is also important, according to our findings, to avoid contact between meat and the hands of workers. Several studies have shown that elimination of carriage in the anterior nares, the principal reservoirs of *S. aureus*, reduces the incidence of *S. aureus* infection (9). Because the surfaces of beef are contaminated with the same type of *S. aureus* as the arms of workers, establishing contamination of the surface of beef carcasses with *S. aureus* on the slaughtering line can be the indicator of work hygiene among workers on the slaughtering line.

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VIR ONESNAŽENJA MLETEGA MESA ZA PROIZVODNJO MESNIH IZDELKOV Z BAKTERIJO STAFILOKOKUS AUREUS

B. Podpečan

Povzetek: V obratu za klanje govedi in predelavo mesa smo ugotavljali vire kontaminacije klavnih trupov in mletega mesa, pripravljenega iz mesa klavnih trupov, in najpomembnejša mesta v proizvodnem procesu, kjer prihaja do kontaminacije.
Odvzeto je bilo skupaj 250 brisov površine govejih klavnih trupov. Vzorci so bili odvzeti na petih različnih mestih na trupu. Bakterijo *S. aureus* smo izolirali na prsih iz 78 % (39/50) vzorcev, na prednji nogi iz 62 % (31/50) vzorcev, na trebušni steni iz 58 % (29/50) vzorcev, na stegnu iz 14 % (7/50) vzorcev in na vratu iz 10 % (5/50) vzorcev. Roke delavcev so pomemben vir kontaminacije mesa. *S. aureus* smo ugotovili pri 50 % (12/24) vzorcev, odvzetih z rok delavcev pred začetkom dela, in pri 58,33 % (14/24) vzorcev, odvzetih z rok delavcev potem, ko so obdelali pet klavnih trupov.
Za diferenciacijo posameznih izolatov bakterije *S. aureus* smo uporabili metodo RAPD-PCR in štiri različne začetne oligonukleotide OPJ5, OPJ6, E7, E8. Ugotovili smo štiri različne genotipe bakterij. Pri tipizaciji izolatov z začetnim nukleotidom OPJ 5 smo ugotovili zvezo med sevi bakterije *S. aureus* z rok, opreme in pribora in sevi s klavnega trupa. Z genotipizacijo z začetnim oligonukleotidom E 8 smo dokazali povezavo med mletim mesom, rokami delavcev, priborom in klavnim trupom. Ker so področja klavnega trupa kontaminirana z enakim tipom bakterije *S. aureus* kot roke delavcev, je ugotavljanje kontaminacije površine klavnih trupov z bakterijo *S. aureus* na klavni liniji lahko pokazatelj higiene dela delavcev.
Glavni vir kontaminacije mletega mesa so roke delavcev (pred začetkom dela je bilo 50 % vzorcev, po obdelavi petih klavnih trupov pa 58,33 % vzorcev kontaminiranih z bakterijo *S. aureus*) in kontaminirana površina klavnega trupa (44 % brisov s površine klavnega trupa je bilo kontaminiranih z bakterijo *S. aureus*).
Za kontaminacijo mletega mesa je kontaminirana površina klavnega trupa odločilna, ker gre tako meso v mletje.

Ključne besede: živali, domače; živali, divje; carnivora; predatorsko vedenje; naravni viri, varovanje-ekonomija; podatki, zbiranje; Slovenija