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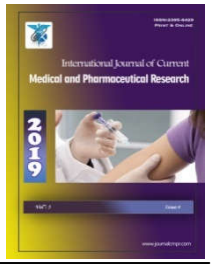

BACTERIOLOGICAL PROFILE AND PHENOTYPIC SIGNS OF FOOD-PROCESSING MICROBIOTA

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ABSTRACT

Objectives: This study was undertaken to isolate and investigatebacteriological profile and phenotypic signs of food-processing microbiota strains including to Escherichia, Klebsiella, Enterobacter, Staphylococcus, Pseudomonas, Citrobacter and Serratia genera. Strains were isolated during hygienic monitoring twomilk-processing factoriesfor the period from December 2013 to November 2014.

Methods: Swabbingwas done in field studies according food safety management protocols in industryfor enumerated contamination by microorganisms and isolation of strains of microbiota.Identification of microorganism was done with culture methods followed by PCR verification. Phenotypic features was studied in vitro with standard biochemical and microbiological methods according good laboratory practice.

Results: Bacterial isolates included to Escherichia, Klebsiella, Enterobacter, Citrobacter, Serratia, Staphylococcus and Pseudomonas genera. Gram-positive cocci account for 20.3 % cases of contamination. In 55 (74.3 %) cases, the pathogens belonged to the family Enterobacteriaceae. Isolate investigations included ability to hemolysis, anti-interferon, anti-lysozyme activity, lecithinase activity, ability to form biofilms in a monoculture of microorganisms. Out of 74 isolates 60 (81.1 %) poses variability of morphological and tinctorial signs.

Conclusions: Gram-negative pathogens and more specifically Escherichia coliaccount for a substantial part of bacterial milk-processing microbiota. Most isolates possessed signs of aggression and ability of biofilmsformation. The obtained experimental data will be used for identification of danger factor accordance with the concept of the quantitative analysis of microbiological risk of food productions.

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INTRODUCTION

Ensuring microbiological safety of foodstuff is one of priority tasks and directed to public health care. According to a number of epidemiologists and microbiologists, phenomenon emergent bacterial infections with food way of transmissioncan be explained with change of phenotypical signs, including the ecological and pathogenetic properties of earlier studied pathogens under the influence of the amplifying anthropogenic factors. At the same time foodstuff and food-processing are represented as new environment or an ecological niche favorable for formation of new phenotypical factors of pathogenicity. Experimental studying of physiology and biochemical properties of isolates of technological environment as a result of hygienic monitoring on the basis of the algorithm offered was the purpose of the study.

MATERIALS AND METHODS

*Methods for recovering microorganisms from solid surfaces.*As conventional swabbing is the recommended method[1], it is commonly used and practically applied in field studies or food safety management protocols in industry.

Methods to analyze microorganisms. Swab samples were investigate using culture methods supplemented by PCR verification[2]. Visual identification of colonies on the selective agar were used for primary identification and further verification of isolate identification was provided by real time PCR with common laboratory procedure. Hemolytic, lecithinase activity and persistence were studied by standard methods[3, 4, 5, 6, 7]. The study of the ability to film formation was carried out according to culture plate method with optical detection[8, 9, 10].

RESULTS

85 bacterial strains were isolated and studied which belong to genera *Escherichia, Klebsiella, Enterobacter, Staphylococcus, Pseudomonas, Citrobacter.*Apart from bacterial pathogens, 60 cases were observed to be contaminated with *Candida, Penicillium and Aspergillus*genera (data not shown). Experimental data of various isolates were depicted in table 1.

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Table 1 Phenotypical sighs of Bacterial isolates

Genera	Phenotypical signs					
	hem-olysis	antilyso zy-me activity	antiinterfe-ron activity	lecithinase activity	biofil mfor-ming	Morphologi -cal and tinctorial properties
Escherichia (19 isolates)	γ	+/-	+/-	+/-	+/-	variable
Klebsiella (9 isolates)	γ	+/-	+/-	+/-	max	variable
Serratia (10 isolates)	γ	+/-	+/-	+/-	+/-	variable
Enterobacter (9 isolates)	γ	+/-	+/-	+/-	+/-	variable
Citrobacter (8 isolates)	γ	+/-	+/-	+/-	+/-	variable
Staphylococcus (15 isolates)	α/β	+/-	+/-	+/-	min	variable/stable
Pseudomonas. (14 isolates)	α	+/-	+/-	+/-	+/-	variable/stable

β-hemolytic activity was found in all studied strains of Staphylococcus aureus and both α- and β-hemolytic activity was demonstrated of Pseudomonas aeruginosa isolates. A number of Staphylococcus spp. with an incomplete hemolytic phenotype have been found in our study. Isolates belong to Enterobacteriaceae family demonstrated γ-hemolytic activity. 100 % of studied strains of Staphylococcus aureus display lecithinase activity.

Anti-interferon and anti-lysozyme activity as persistence factors aimed at inactivating host defense mechanisms have been studied in Staphylococci. Of the 9 studied strains of Staphylococcus aureus, 8display anti-lysozyme activity at a lysozyme concentration of 4 μg / ml or less, 5 strains had anti-interferon activity (interferon concentration 2 units), 3 strains had anti-interferon activity (interferon concentration 1 units). The phenomenon is accordance withgood ability of staphylococcal strains grow on nutrient agar with fuzidin concentration of 0.00015-0.0003 g/l.

The studied strains have a different ability to biofilm formation in a monoculture of microorganisms. All strains of *Klebsiellapneumoniae* have a high degree of film-forming ability according to the *Stepanovic* criterion. Coagulase-negative staphylococcus (*S. haemoliticus, S. sciuri, S.epidermidis*) possessed minimal film-forming ability.
Out of 85 isolates 60 poses variability of morphological and tinctorial signs.

DISCUSSION

It is important to note that the correct choice of methods to isolate and investigate phenotypical properties of bacterial strains[1]is significant. The choice of the recovery method has to be suitable for the type and size of the surface tested for microbiological analysis.

The phenomena of variability of morphological and tinctorial properties of isolates have been noted[11, 12, 13]. Isolates of hospital environments as well as food-processing display atypical signs, such as strong ability to hemolysis, anti-interferon, anti-lysozyme activity, lecithinase activity, ability to form biofilms. On the other hand, recently a number of strains belonging to a class of S. aureus with an incomplete hemolytic phenotype.

In our study, out of 74 isolates 60 (81.1 %) poses variability of morphological and tinctorial signs. Bacteria genera *Escherichia, Klebsiella, Enterobacter, Staphylococcus, Pseudomonas, Citrobacter* and *Serratia* are widespread contaminants isolated duringfood-processing, which can causes both hospital and community-acquired infections[13]. It was reported that a lot of microbial contaminants can survive in a range of solid surfaces, such as plastic, stainless steel, glass, and wood[1]. These surfaces are subject to contamination by microorganisms responsible for the cross-contamination of food by contact with working surfaces. This preventive approach has resulted in the use of microbiological analyses of surfaces as one of the tools to control the hygiene of products and limit contamination risks. The virulence of microbial contaminants is closely associated with a variety of secreted enzymes and toxins produced by the bacteria. Recent studies have demonstrated that hemolysin also participates in the formation of the S. aureus biofilm[13].

CONCLUSION

The obtained experimental data will be used for identification of danger within the concept of the quantitative analysis of microbiological risk of food productions. The increasing prevalence of strains with atypical signs within community environments further increases the dangers of microbial contaminants.

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