Antibiotic Resistance of Microbial Contaminations Isolated from Husbandry Animals and Foodstuffs

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Abstract
In this paper the antibiotic resistance of microbial contaminations isolated from husbandry animals and foodstuffs were investigated. Microorganisms isolated from animals and foodstuffs were contaminations of selective media as MacConkey agar for *Enterobacteriaceae* genera and MRS agar for lactobacilli strains. Microorganisms were isolated and puryfied by agar four ways streak plate method. Identification of isolated microorganisms was done by mass-spectrometry method in MALDI-TOF MS Biotyper. For investigation of antibiotic resistance disc diffusion method by EUCAST was used. In this study Gram-negative and Gram-positive bacteria were identified. The most resistant or multi-resistant bacteria as *Pseudomonas aeruginosa*, *Acinetobacter lwoffi*, *Lysinibacillus sphaericus*, *Staphylococcus aureus* and *Staphylococcus epidermis* were determined. Other identified microorganisms were resistant to one antibiotic or not at all.

Keywords: Antibiotic resistance, microbial contamination, identification, Gram-positive and Gram-negative microorganisms

1. Introduction

Antibiotic resistance is significant health, social and economic problem at this time. Antibiotic resistance of bacteria is biological risk, which increases morbidity and mortality of animals and humans [1, 2].Antibiotic resistant bacteria and drug resistance genes have become an important environmental contamination issue which is receiving an increased attention [3-4]. The antibiotic resistance genes can be transferred between bacteria in the environment through plasmids, integrons and transposons [4, 6, 8]. Keyser et al. [9] noted that in recent year, accumulating problems with resistant bacteria, leading to predictions that we are back the period before the discovery of antibiotics. Infections caused by resistant strains of microorganisms causing costly treatment of animals and humans. These infections prolong the pathological condition and if they are not treated with the right antibiotics they can increase mortality [10].
The main aim of our research was study of some isolated bacterial strains.

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2. Materials and methods

Collection of samples

The samples of microbial contaminations were obtained from contaminated agars in four years from 2010 to2013. Microorganisms were isolated from various husbandry and foodstuffs sources as ducks, gooses, fishes, rabbits, bees, cheeses, sheep cheeses, more kind of sheep cheeses and pollen. The samples were collected by sterile swab kit (Copan Inovation, Italy) and inoculated into the medium to laboratory of Department of

Microbiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra.

Cultivation of microorganisms

Microorganisms were cultivated on MacConkey (Biomark, Pune) agar and MRS agar (Biolife, Italy) as microbial contamination. Cultivations were done at 37°C during 24 hours for microorganisms which grew on MacConkey agar and 37°C during 48-72 hours for microorganisms which grew on MRS agar. The purifying of all microorganisms was done by four ways streak plate method after the first cultivation. There were used Chromogenic colifrom agar (Oxoid, England) and URI Select IV (Biolife, Italy) for purifying of microorganisms that contaminate MacConkey agar. Microorganisms which contaminated MRS agar were purified in MRS agar (Biolife, Italy) again. Every these steps of recultivation were done at the same conditions.

Identification of microorganisms by MALDI TOF MS Biotyper

There was used for identification of microorganism's matrix-assisted laser desorption/ionization time-of-light (MALDI TOF MS) (Brucker Daltonics, Germany).

There was used MALDI TOF MS bacterial analysis, cells from a single colony of fresh overnight culture for each isolate to prepare samples according to the manufacturer's recommendations for microorganism profiling ethanol-formic acid extraction procedure. Each sample spot was overlaid with 2 µL of matrix solution (satured solution of α-cyano-4-hydroxy-cinnamic acid in 50 % acetonitrile with 2.5 % trifluoroacetic acid) and again air-dried for 15 min. To identify microorganisms, the raw spectra obtained for each isolate were imported into Biotyper software, version 2.0 (Brucker daltonics, Germany), and analyzed without any user intervention.

Susceptibility testing

Testing of the antimicrobial susceptibility of obtained microorganisms was performed by EUCAST disk diffusion methodology, version 3.0 from April 2013 [11]. Antibiotics discs as tetracycline (TET) 30 µg/disc, chloramphenicol (CHL) 30 µg/disc, ampicillin (AMP) 10 µg/disc, vancomycin (VAN) µg/disc, piperacillin (PIP) 30 µg/disc, gentamicin (GEN) 10 µg/disc, erythromycin (ERY) µg/disc, meropenem (MEM) 10 µg/disc and amikacin (AMI) 30 µg/disc were used in this experiment. For evaluation of inhibition zones around the discs, breakpoint tables for interpretation of MICs and zone diameters [12] established by EUCAST was used.

3. Results and discussions

Many kinds of microbial strains were isolated and identified in this experiment. We found that the most microbial strains isolated were *Pseudomonas* species, *Acinetobacter* species and *Staphylococcus* species.

The other bacterial strains were isolated with less frequency. The yeasts were also isolated with less frequency. The most contaminated samples were sheep's cheese and sheep's whey.

Gram-negative isolates

There were isolated and identified *Pseudomonas aeruginosa*, *P. fulva*, *P. oryzihabitans*, *P. taetrolens*, *Moraxella osloensis*, *Acinetobacter lwoffii*, *A. genomospecies*, *A. radioresistens* and *A. baumannii* from this group.

Pseudomonas aeruginosa were isolated from McConkey agar (McC) and MRS agar. Gastrointestinal tracts (GIT) of ducks and honey bees were sources of *Pseudomonas aeruginosa*. *P. aeruginosa* isolated from GIT ducks were cultivated on McC agar with another *Enterobacteriaceae* strains together and they were resistant to three antibiotics, tetracycline, chloramphenicol and natural resistant to ampicillin.

P. aeruginosa isolated from GIT of honey bees were cultivated on MRS agar with other lactobacilli together and they were resistant to vancomycin only.

Pseudomonas fulva were isolated from GIT of gooses which showed good growth on McC agar and URI Select IV agar (URI). These microorganisms showed the resistance to ampicillin.

Pseudomonas oryzihabitans were isolated from sheep's cheese (oštiepok) on McC and URI agar. These ones were also resistant to ampicillin.

Pseudomonas taetrolens grow on McC agar were isolated from the GIT of honey bees. These ones were resistant to piperacillin.

Moraxella osloensis and *Enterobacteriaceae* strains were isolated from pollen samples on McC and URI agar. Susceptibility testing showed resistance to piperacillin.

There were three species (*A. lwoffii*, *A. genomospecies* 3, *A. baumannii*) of *Acinetobacter* sp. isolated from sheep milk products and one species (*A. radioresistens*) isolated from pollen samples.We found positive effect of MRS agar on growth of all isolates of *Acinetobacter* sp. We used this MRS agar for lactobacilli strains cultivation). Also these microorganisms grew on URI agar. *Acinetobacter baumannii* isolated from sheep's whey proliferated on URI agar at 20°C only. *Acinetobacter* was *A. lwoffii* was the most resistant and multi-resistant.

Detail information about tested microorganisms are described into the Table 1.

Gram-positive isolates

There were only *Micrococcus luteus* from the Gram-positive group of microorganisms isolated from GIT of fishes and gooses. They were contaminated onMcC and URI agars and they were resistant to ampicillin.

Lysinibacillus sphaericus were isolated from sheep's whey (žinčica) as contamination of MRS agar. These microorganisms showed a multi-resistance to erythromycin, vancomycin, gentamicin and meropenem.

Many bacterial strains from *Staphylococcus* sp. were isolated from different samples as sheep's whey, sheep's cheese, pollen, GIT of bees and rabbits. These microorganisms showed good growth on McC, MRS and URI agars. Also these microorganisms showed resistance to different antibiotics. The highest resistance and multi-resistance showed *Staphylococcus aureus* and *Staphylococcus epidermis* isolated from sheep's whey. Other *Staphylococcus* strains were resistant to ampicillin, piperacillin and amikacin only.

Bacterial strain as *Staphylococcus hominis, Streptococcus salivarius* and *Leuconostoc mesenteroides* were not resistant to antibiotics which we used in this experiment.

We also detected the bacterial strain *Bacillus cereus* that was resistant to ampicillin and it was isolated from sheep's cheese cultivated on chromogenic coliform agar. More detail information is described into the Table 1.

Yeasts

We isolated two yeasts as *Candida albicans* (anamorf) and *Pichia fermentas* (teleomorf) from this group of microorganisms. These yeasts showed very good growth on MRS agar only. Yeasts are resistant to bacterial antibiotics. More detailed information is described into the Table 1.

Discussions

The antibiotic resistance of Pseudomonas aeruginosa is described in the study of Rubin et al., [13] They found out an antibiotic resistance of *Pseudomonas aeruginosa* in their study and these bacterial strains were isolated from dogs GIT. There were tested a lot of antibiotics in this study. They found 100 % resistance to ampicillin. *Pseudomonas aeruginosa* are inherently resistant to ampicillin. This confirms the results of EUCASTs report. Rubin et al. [13] tested tetracycline and chloramphenicol and they determined high level of resistance to these antibiotics. The resistance to tetracycline was 98 % and resistance to chloramphenicol was 100 %. They didn't test vancomycin in contrast with our study.

Tognim et al. [14] tested an antibiotic resistance of *Acinetobacter* species in their study. They determined the resistance to meropenem and gentamicin and their samples were obtained from medicinal centers in Brazil during five years. These authors didn't test ampicillin and erythromycin for *Acinetobacter* sp.

Fernández-Cuenca et al. [15] found out very similar results. They also determined resistance to meropenem but their samples were obtained from environment of hospital.

Deccache et al. [16] determined the resistance to quinolone antibiotics in *Acinetobacter baumannii*. These results are in contrast with our results.

Many researchers and authors have interested about an antibiotic resistance of *Staphylococcus* sp. because these ones are very important bacteria from the clinical microbiology point of view.

There are many studies confirming the resistance and the multi-resistance to different kinds of antibiotics.

In the present we have some knowledge about the resistance to methicillin, mupirocin, erythromycin and to fluoroquinolones antibiotics [17-21].

In accordance with our study Chaves et al. [22] also isolated *Bacillus cereus* from milk products and they tested *B. cereus* against four antibiotics.

They determined resistance to vancomycin and tetracycline.

In the present there is not any experiment or results about an antibiotic resistance of other bacterial strains. Because of this it is very difficult to compare our other result.

Table 1. Antibiotic resistance of microorganisms which were isolated as microbial contamination

Microorganisms	Culture medium	Sources	Antibiotics
Gram-negative			
Pseudomonas aeruginosa	McC	Ducks	TET, CHL, AMP^{P}
	MRS	Bees	VAN
Pseudomonas fulva	McC/URI	Gooses	AMP
Pseudomonas oryzihabitans	McC/URI	Sheep's cheese (oštiepok)	AMP
Pseudomonas taetrolens	McC	Bees	PIP
Moraxella osloensis	McC/URI	Pollen	PIP
Acinetobacter lwoffii	MRS	Sheep's cheese (parenica)	ERY
	MRS	Sheep's whey (žinčica)	ERY, AMP, GEN, MEM
	URI	Sheep's cheese (bryndza)	AMP
Acinetobater genomospecies 3	MRS	Sheep's cheese (parenica)	-
	URI		ERY
Acinetobacter radioresistens	URI	Pollen	-
Acinetobacter baumannii	URI*	Sheep's whey (žinčica)	AMP^{P}
Gram-positive			
Micrococcus luteus	McC/URI	Fishes, gooses	AMP
Lysinibacillus sphaericus	MRS	Sheep's whey (žinčica)	ERY, VAN, GEN, MEM
Staphylococcus aureus	MRS	Sheep's whey (žinčica)	ERY, VAN, GEN, MEM
Staphylococcus epidermis	CHR	Sheep's cheese (parenica)	AMP
	MRS	Sheep's whey (žinčica)	ERY, AMP, GEN, MEM
	MRS	Sheep's whey (žinčica)	AMP
Staphylococcus succinus	McC/URI	Pollen	AMP
Staphylococcus vitulinus	McC	Bees	PIP
		Bees	PIP, AMI
		Rabbits	PIP
Staphylococcus warneri	McC/URI	Sheep's whey (oštiepok)	AMP
Staphylococcus hominis	McC/URI	Work contamination	-
Streptococcus salivarius	MRS	Work contamination	-
Leuconostoc mesenteroides	MRS	Sheep's whey (oštiepok)	-
Bacillus cereus	CHR	Sheep's whey (oštiepok)	AMP
Yeasts			
Candida lambica (anamorfa) *Pichia fermentas (teleomorfa)*	MRS	Sheep's whey (žinčica)	Resistant against bacterial antibiotics

Legend: McC – MacConkey agar, MRS – agar for lactobacilli, URI – chromogenic agar for urinary tract pathogens, CHR – chromogenic coliform agar, TET – tetracycline, CHL – chloramphenicol, AMP – ampicillin, VAN – vancomycin, PIP – piperacillin, ERY – erythromycin, GEN – gentamicin, MEM – meropenem, AMI – amikacin, ^{P} – natural resistance, * - growth at 20 °C

4. Conclusions

On the basis of our result we can say that, many kinds of bacterial strains can grow and contaminate the selective media for selective cultivation of bacteria. We found out that bacterial strains as *Pseudomonas aeruginosa, Acinetobacter lwoffii, Lysinibacillus sphaericus, Staphylococcus aureus, Staphylococcus epidermis* expressed characters of resistance and multi-resistance to several types of tested antibiotics.

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References

1. EFSA, Foodborne antimicrobial resistance as a biological hazard, Draft Scientific Opinion of the Panel on Biological Hazards (Question No EFSA– Q-2007-089), Draft endorsed on 6 March, 2008.
2. Horská, E., Yespolov, T.I. et al. 2013. Sustainability in Business and Society: Global Challenges – Local Solutions. Krakow: Wydawnictwo Episteme, 2013, 166 p. ISBN 978-83-7759-015-7.
3. Kummerer, K. 2004. Resistance in the environment. Journal of Antimicrobial Chemotherapy, 54, 311-320.
4. Pruden, A., Pei, R., Storteboom, H., Carlson, K. H. 2006. Antibiotic resistance genes as emerging contaminants: studies in northern Colorado. Environmental Science and Technology, 40, 7445-7450.
5. Sapkota, A. R., Curriero, F. C., Gibson, K. E., Schwab, K. J. 2007. Antibiotic-resistant Enterococci and fecal indicators in surface water and groundwater impacted by a concentrated swine feeding operation. Environmental Health Perspectives, 115, 1040-1045.
6. Pang, Y., Brown, B. A., Steingrube, B. A. 1994. Acquisition of gram-positive tetracycline resistance genes in *Mycobacterium* and *Streptomyces* species. Antimicrobial Agents and Chemotherapy, 38, 1408-1412.
7. Schwarz, S., Chaslus-Dancla, E. 2001. Use of antimicrobials in veterinary medicine and mechanisms of resistance. Veterinary Research, 32, 201-225.
8. Nordmann, P., Poirel, L. 2005. Emergence of plasmid-mediated resistance to quinolones in *Enterobacteriaceae*. Journal of Antimicrobial Chemotherapy, 56, 463-469.
9. Keyser, P., Elofson, M., Rossel, S., Wolf-Watz, H. 2008. Virulence blockers as alternatives to antibiotics: type III secretion inhibitors against Gram-negative bacteria. Journal of Internal Medicine, 264(1), 17-29.
10. Witte, W. 2006. Selective pressure by antibiotics use in livestock. International Journal of Antimicrobial Agents, 16, 19-24.
11. European Committee on Antimicrobial Susceptibility Testing (EUCAST). 2013. Antimicrobial susceptibility testing: Eucast disk diffusion method, version 3.0 from April 2013.
12. European Committee on Antimicrobial Susceptibility Testing (EUCAST). 2014. Breakpoint tables for interpretation of MICs and zone diameters, version 4.0 valid from 2014-01-01.
13. Rubin, J.,Walker, R. D., Blickenstaff, K., Bodeis-Jones, S., Zhao, S. 2008. Antimicrobial resistance and genetic characterization of fluoroquinolone resistance of *Pseudomonas aeruginosa* isolated from canine infections. In Veterinary microbiology, 131, 164-172.
14. Tognim, M. C. B., Andrade, S. S., Silbert, S., Gales, A. C., Jones, R. N., Sader, H. S. 2004. Resistance trends of *Acinetobacter* spp. in Latin America and characterization of international dissemination of multi-drug resistant strains: five-year report of the SENTRY antimicrobial surveillance program. International journal of infection diseases, 8, 284-291.
15. Fernández-Cuenca, F., Gómez-Sánchez, M., Rodrígez-Bano, J., Martínez-Martínez, L., Vila, J., Bou, G., Pascual, A. 2012. Epidemiological and clinical features associated with colonisation/infection by *Acinetobacter baumannii* with phenotypeic heterogenous resistance to carbapenems. International journal of antimicrobial agents, 40, 235-238.
16. Deccache, Y., Irenge, L. M., Savov, E., Ariciuc, M., Macovei, A., Trifonova, A., Gergova, I., Ambroise, J., Vanhoof, R., Gala, J. L. 2011. Development of a pyrosequencing assay for rapid assessment of quinolone resistance in *Acinetobacter baumannii* isolates. Journal of microbiological methods, 86, 115-118.
17. Kresken, M., Hafner, D., Schmitz, F. J., Wichelhasu, T. A. 2004. Prevalence of mupirocin resistance in clinical isolates of *Staphylococcus aureus* and *Staphylococcus epidermis*: results of the antimicrobial resistance surveillance study of the PaulErlich-Society fo Chemotherapy, 2001. International journal of antimicrobial agents, 23, 577.581.
18. Sevin, E. – Sevin-Larmaraud, O. – Legrand, P. 1999. Approche moléculaire de la résistance á la métilinne de *Staphylococcus aureus.* Revue Francaise des Laboratories, 315, 25-31.
19. Westh, H., Knudsen, A. M., Gottschau, A., Rosdahl, V. T. 1991. Evolution of *Staphylococcus aureus* resistance to erytromycin in Denmark, 1959 to 1988: comparison with erytromycin-susceptible strains. Journal of hospital infection, 18, 23-34.
20. Rio, Y., Jurin, F., Didion, J., Staal, A. 2005. Sélection in vitro de mutants résistant de *Staphylococcus aureus* en présence de flouroquinolones par la méthode des passages successif. Antibiotiques, 7, 191-195.
21. Berger-Bächi, B. 1995. Factors affecting methicillin resistance in *Staphylococcus aureus*. International journal of antimicrobial agents, 6, 13-21.
22. Chaves, J. Q., Pires, E. S., Vivoni, A. M. 2011. Genetic diversity, antimicrobial resistance and toxigenic profiles of *Bacillus cereus* isolated from food in Brazil over three decades. International journal of food microbiology, 147(1), 12-16.