SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D.)

Analysis of the ß-lactamases of borderline methicillin-resistant *Staphylococcus aureus* strains

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The Examination takes place at the Discussion Room of the Department of Immunology (ÉTK 2.209), Medical and Health Science Center, University of Debrecen
11th April, 2012

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The Ph.D. Defense takes place at the Lecture Hall of the 1st Department of Medicine, Institute for Internal Medicine, Medical and Health Science Center, University of Debrecen
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1. INTRODUCTION

1.1. ß-lactam-resistance in *Staphylococus aureus*

The Gram-positive *Staphylococcus aureus* is one of the most important facultative human pathogenic bacteria. The first antibiotic, the ß-lactam ring containing penicillin was also introduced to treat staphylococcal infections. Few years later the first ß-lactamase producing penicillin-resistant strains appeared, so new antibiotics had to be found or developed. The infections caused by such strains were treated with penicillin applied in combination with ß-lactamase inhibitors (clavulanic acid, sulbactam) or with semisynthetic penicillinase-resistant penicillins (PRPs) like methicillin, oxacillin, nafcillin, cloxacillin. With the spread of nosocomial and lately community-associated strains becoming methicillin- or oxacillin-resistant not because of ß-lactamase production, the application of other antibiotics (like glycopeptides) were favoured, but these antibiotics are the last defense lines against multiresistant strains.

The ß-lactam antibiotics act on the transpeptidation step of the cell wall synthesis. It is an extracellular step which follows the incorporation of a new cell wall unit (N-acetylmuramic acid – N-acetylglucosamine disaccharide, an L-Alanine – D-Glutamic acid – L-Lysine – D-Alanine – D-Alanine pentapeptid is attached to the N-acetylmuramic acid, and a pentaglycine part attached to the L-Lysine) into the cell wall (transglycolisation). Cross bridges are produced by the transpeptidase domain of penicillin-binding proteins (PBPs) with the removal of the terminal D-Alanine from the pentapeptide and the attachment of the pentaglycine part of an other cell wall unit to the D-Alanine of the remaining tetrapeptide. The structure of the ß-lactam ring is similar to that of the D-Alanine-D-Alanine dipeptide, so such antibiotics are recognized and bound by PBPs, but they cannot be hydrolysed.

The PRP-resistance of *Staphylococcus aureus* can be traced back to various causes. It can be the consequence of the production of PBPs with decreased penicillin-binding ability like the *mecA* encoded PBP2a in the case of MRSA (methicillin-resistant *Staphylococcus aureus*) strains. The *mecA* is located in the neighbourhood of many other resistance genes in SCC*mec* (staphylococcal cassette chromosome *mec*), which is an important pathogenicity island. Lower antibiotic-resistance can be caused by ß-lactamases usually with a Serine in their active site. The ß-lactam ring splitted in the CO-N bound creates a penicilloyl enzyme with the hydroxyl group of this Serine. The difference between PBPs and enzymes is that the

latter hydrolyses the antibiotic and regenerates its hydroxyl group with the incorporation of water. The enzymes are mostly secreted but membrane-bound forms also exist.

1.2. The characters of borderline methicillin-resistant *Staphylococcus aureus* (BORSA) strains

The BORSA (borderline methicillin-resistant *Staphylococcus aureus*) strains show an intermediate oxacillin-resistance compared with susceptible and MRSA strains. The elevated MIC (minimal inhibitory concentration) is mostly the consequence of the hyperproduction of a ß-lactamase (BlaZ penicillinase), but the introduction of the ß-lactamase gene into susceptible strains and the hyperproduction of the enzyme is not enough to elevate the oxacillin MIC. Only strains typable with phages belonging to phage group 94/96 (V.) turned out to be borderline oxacillin-resistant after the transformation of the 17.2 kDa *blaZ*-bearing pBW15 plasmid. If this plasmid was lost the strains became susceptible to PRPs, so the gene of the resistance-causing factor had to be on the plasmid.

Massidda and co-workers showed that after methicillin-induction beside BlaZ (32 kDa) a 31-kDa enzyme appeared in the membrane fractions of the examined strains. This enzyme was able to hydrolyse not only nitrocefin and penicillin, but also methicillin, oxacillin and PADAC (nitrocefin and PADAC – Pyridinium-2-Azo-p-Dimethylaniline Chromophore are chromogenic cephalosporins, which are applied to prove the existence of ß-lactamases, because as they are hydrolysed their colour is changed from yellow to red or from purple to yellow, respectively). They thought that it could be a new methicillinase/oxacillinase enzyme characteristic to BORSA strains.

This hypothesis was confirmed, because after the transformation of a *blaZ*-bearing plasmid into *Escherichia coli*, the produced ß-lactamase was not able to hydrolyse PRPs, so either because of the differences in the genetic background or because the gene of the oxacillinase was not present on the plasmid the oxacillinase was not expressed in *E. coli*. It was hypothesized that this new oxacillinase was developed by the mutation of BlaR1 (a transmembrane protein involved in the regulation of the expression of BlaZ), but no evidence was found.

2. OBJECTIVES

In Gram-negative Enterococci an enzyme was described which was able to hydrolyse oxacillin. Dr. Orietta Massidda and co-workers found an enzyme in borderline methicillin-resistant *Staphylococcus aureus* (BORSA) strains, which also was able to hydrolyse oxacillin and methicillin, appeared after methicillin induction in the membrane fraction, its molecular weight was 31 kDa (the MW of BlaZ penicillinase is 32 kDa), and it was not transmissible to *E. coli* with the transformation of a *blaZ* bearing plasmid. Our final goal was the identification of this oxacillinase.

The main objectives of our work were as follows:

1. To collect Staphylococcus strains from different sources and to identify the BORSA strains based on their oxacillin MIC.

2. The characterization of the collected BORSA strains, i.e. determining the possible PBP2a production, phage typing, determining their ß-lactamase activity.

3. Detection and identification of the hypothetic methicillinase/oxacillinase by proteomic methods.

4. Detection the possible differences among the ß-lactamases of strains with different origin (human isolates and strains isolated from bovine mastitis).

3. MATERIALS AND METHODS

3.1. Collecting human and bovine strains

Most of the investigated human isolates derived from the strain collection of the Department of Pharmacology and Pharmacotherapy, Medical and Health Science Center, University of Debrecen. In this collection the reference strains were provided by Dr. Douglas S. Kernodle (Vanderbilt University, Nashville, Tennessee., USA). The 56 human isolates (52 *Staphylococcus aureus*, 4 *Staphylococcus epidermidis*) were provided by the Department of Microbiology, Medical and Health Science Center, University of Debrecen. The penicillin, methicillin, and oxacillin MIC values of all those strains were determined earlier alone or in combination with sulbactam or clavulanic acid by macrodilution method according to the guidelines of Clinical and Laboratory Standards Institute (CLSI, former NCCLS – National Committee for Clinical Laboratory Standards). Among those strains five proved to be BORSA. Two other human isolates (a36 and a53) were sent by Dr. Marina Mingoia for Dr. Orietta Massidda's intercession from the strain collection of the Institute of Microbiology, University of Ancona Medical School, Italy. Two *Staphylococcus aureus* and four *Staphylococcus epidermidis* strains were provided by Dr. Ferenc Rozgonyi (Semmelweis University, Department of Medical Microbiology, Budapest). 28 strains isolated from animal infections and foods were sent by Dr. Éva Kaszanyitzky and Dr. Szilárd Jánosi (Central Veterinary Institute, Budapest).

3.2. MIC determination by the macrodilution method

The classification of the strains was based on the MIC determination by macrodilution method according to the guidelines of CLSI. As our work was the continuation of the experiments of Dr. Orietta Massidda and her co-workers, we used their criteria for the selection of BORSA strains (oxacillin MIC was 1-2 μg ml^{-1}, and it decreased under 1 μg ml^{-1} to the effect of ß-lactamase inhibitors).

3.3. Detection of PBP2a

The latex agglutination test (PBP2a latex agglutination test, Oxoid, Basingstoke, UK) was used to determine the PBP2a production of the human isolates according to the manufacturer's instructions. In the cases of the other strains the presence of *mecA* was determined by PCR at the time of their isolation

3.4. Phage typing

Phage typing was performed with the international phage set of the National Public Health and Medical Officer Service, according to the guidelines of WHO (World Health Organization), by the phage laboratory in Debrecen. The applied phage groups were: phage group I.: 29, 52, 52A, 79, 80; phage group II.: 3A, 3C, 55, 71; phage group III.: 6, 42E, 47, 53, 54, 75, 77, 83A, 84, 85; phage group IV.: 42D; phage group V.: 94, 96; mixed phage group: 81, 95, 187; MRSA phages: 616, 617, 618, 620, 622, 623, 625, 626, 629, 630; Bovine phages: 116, 102, 107, 117, 118, 119, Ae1, 78.

3.5. Isolation of bacterial membranes

Bacteria were grown in 1% pH 7.4 CY (casein-yeast) broth supplemented with 0.5 μg ml^{-1} methicillin for 16 hours at 37ºC. Harvested and washed cells were disrupted by incubation in 1 mg ml^{-1} lysozyme solution followed by osmotic lysis, then membrane was isolated with serial centrifugation. When the yield of that protocol was not satisfactory cells were sonicated by Branson Sonifier 250, or in the case of strain a53 X-Press was used (X 25, AB Biox, Goteborg, Sweden). X-Press was performed by Dr. Tamás Emri (Department of Microbial Biotechnology and Cell Biology, University of Debrecen).

3.6. Preparation of bacterial supernatants

50 ml aliquots of supernatants were filtered via a Millipore sterile filter (0.45mm, Millipore, Bedford, USA) then concentrated with ultrafiltration (PM10 membrane with 10000 Da cut off or YM5 membrane with 5000 Da cut off, Amicon, Millipore, Bedford, USA), with continuous stirring and application of nitrogen gas at a pressure of 2 atmospheres, and finally centrifuged with Centricon YM-3 Centrifugal Filter Units (Millipore, Bedford, USA).

3.7. Measurement of Oxacillin-Hydrolyzing Activity (Bioassay)

50 μl of culture supernatants or membrane fractions were mixed with 50 μl 80 μg ml^{-1} oxacillin. After 30 min of incubation, the decreased oxacillin concentration was measured by agar diffusion method using *Bacillus subtilis* ATCC 6633 as a test strain. The oxacillin-hydrolyzing activity was evaluated after incubation at 37 °C for 24 h by measuring the inhibition zones.

3.8. Detection of ß-lactamases with SDS-PAGE

The membrane-bound and the extracellular proteins of the strains were separated by 1-D than 2-D gel electrophoresis. In the latter case previous to the separation of the proteins according to their molecular weights isoelectric focusing was performed: in the case of membrane-bound proteins the cup loading, in the case of the extracellular proteins the classic technique was applied using 2% CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate), 50 mM DTT (dithiotreitol), 0.2% Bio-Lyte 3/10 ampholyte, 0.002% bromophenol blue containing sample buffer supplemented with 7 M urea and 2 M thiourea or only with 8 M urea, respectively.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the protocol described by Laemmli.

After SDS-PAGE one of the two parallel gels was soaked in a renaturating buffer containing 1% Triton X-100 and 0.1 mM zinc-sulphate for 8-10 h at 37°C. Enzymatic activity was detected directly by placing filter paper strips soaked in 1 mg ml^{-1} nitrocefin solution on the appropriate areas of the gels corresponding to areas where we detected activities on 1-D SDS-PAGE previously. After the detection and marking of the ß-lactamase dots or bands the gels were stained with Coomassie Brilliant Blue G-250 (Blue Silver). The parallel gels were stained with Coomassie Brilliant Blue G-250 and appropriate spots were cut out from the gels.

3.9. Mass spectrometric analysis of ß-lactamases

The identification of the proteins was made by MALDI-TOF (matrix assisted laser desorption ionization–time of flight) detector or by LC-MS/MS (liquid chromatography–tandem mass spectrometry), implemented by the Proteomics Research Group of Dr. Katalin Folkl Medzihradszky, or by the workers of Kromat Ltd. (Biological Research Center of the Hungarian Academy of Sciences, Szeged).

3.10. Measurement of ß-lactamase activity by spectrophotometric method

The antibiotic-hydrolyzing activity of proteins was measured by Perkin-Elmer λ 12 UV/VIS spectrophotometer. In each case the temperature was set at 37°C, and the reactions were followed for 10 minutes. The blank was composed of 0.1 ml Na-phosphate buffer (0.05 M, pH 7.0) and 0.9 ml antibiotic stock solution, the samples were created from 0.1 ml enzyme (or 0.1 ml 10 mg ml^{-1} lysozyme stock solution) and 0.9 ml antibiotic stock solution. All the antibiotic stock solutions (penicillin G, oxacillin, cefalorodine, cefamandole, cefoperazone) except nitrocefin were applied in a ten-fold dilution. The spectrophotometric analyses were

performed at the following wavelengths: nitrocefin: 486 nm, penicillin G: 233 nm, oxacillin: 260 nm, cefalorodine: 260 nm, cefamandole: 274 nm, cefoperazone: 273 nm. The enzyme activity was calculated based on the following formula:

$(dA/dt) \times (1000\ l/\varepsilon) \times (V_s/1000)\ l \times D_e$, where

dA: change in absorption; dt: change in time; ε: extinction (absorption) coefficient; V_s: volume of the sample (ml); D_e: dilution of the enzyme. The ε values ($mM^{-1}cm^{-1}$): nitrocefin: 16.7, penicillin G: 0.94, oxacillin: 0.45, cefalorodine: 10.2, cefamandole: 10.3, cefoperazone: 9.

3.11. Applied antibiotics and substrates

The following compounds were used in our experiments: methicillin (Bristol Laboratories, Paris, France), penicillin G (TEVA, Debrecen, Hungary), oxacillin (Sigma-Aldrich, St. Louis, USA), cefaloridine (Sigma-Aldrich, St. Louis, USA), cefamandole (Sigma-Aldrich, St. Louis, USA), cefoperazone (Sigma-Aldrich, St. Louis, USA). clavulanic acid (Sigma-Aldrich, St. Louis, USA), sulbactam (Sigma-Aldrich, St. Louis, USA), nitrocefin (Oxoid, Basingstoke, UK), lysozyme (Sigma-Aldrich, St. Louis, USA).

4. RESULTS AND DISCUSSION

4.1. Determination of the MIC values of the strains provided by the Central Veterinary Institute and by the Semmelweis University, Department of Medical Microbiology

The oxacillin MIC values of the isolates were determined either alone or in a combination with clavulanic acid or sulbactam. Among the strains collected by the Central Veterinary Institute 4 strains isolated from bovine mastitis proved to be BORSA (19599/3A, 19599/3B, 7789/47, 7789/98). All the strains isolated at the Semmelweis University were oxacillin-susceptible. So together with the BORSA strains found in the strain collection of the Department of Pharmacology and Pharmacotherapy (VU94, 822, 14287/1, 9800, 9989) and with the strains provided by Dr. Massidda and Dr. Mingoia (a36, a53), totally 11 strains were investigated.

4.2. Detection of PBP2a

PBP2a detection was performed in the cases of the human isolates. The results were in correlation with the expectations based on the MIC values. The methicillin- and oxacillin-resistant strains produced PBP2a, and the susceptible or borderline resistant strains did not. There was only one exception, the strain 822, which was classified as a BORSA strain according to its MIC values, but it proved to be a PBP2a producer. The presence of *mecA* was confirmed by PCR. Probably the PBP2a cannot function properly, that i, why this strain had a low oxacillin MIC value.

In the case of the strains provided by the Central Veterinary Institute and by Dr Massidda and Dr. Mingoia the absence of *mecA* was determined at the time of the isolation of the strains.

4.3. Phage typing

All the human isolates except the *mecA* bearing strain 822 were typable with group V. phages. This strain was not typable with any other phages. Two of the bovine isolates (19599/3A, 19599/3B) were also typable with group V. phages, but interestingly these strains were not typable with the bovine phages, so they might had had transferred from human to cow by reverse zoonoses. The other two bovine isolates (7789/47, 7789/98) were typable with bovine phages, and strain 7789/98 with group V. phages, too.

4.4. Measurement of the oxacillinase activity

The exact oxacillinase activity of the extracellular and the membrane-bound proteins of the strains were also determined. The oxacillin-hydrolysing activity could not be determined by spectrophotometric measurement. We had to apply biological titration. Oxacillin was hydrolysed at a $10^{-3} – 10^{-4}$ μmol minute^{-1}mg total protein^{-1} activity. All the strains produced ß-lactamase constitutively and the activity (the rate of enzyme production) could be increased (although not significantly) by culturing the strains in the presence of methicillin. The slowest hydrolyser was the bovine strain 7789/47, which was not typable with group V. phages. It correlates with the previous findings that only BORSA strains typable with group V. phages possessed significant oxacillinase activity. Somewhat contradictory to this theory that *mecA* harbouring strain 822 which was not typable with any kind of phages, possessed one of the highest enzymatic activities.

4.5. Determination of the molecular weight ranges of ß-lactamases by 1-D SDS-PAGE

First the ranges of the molecular weights of ß-lactamases were determined to concentrate only on the appropriate region(s) during 2-D gel electrophoresis. On the gels two regions were detected with ß-lactamase activity: the 30-34 kDa and the 10-14 kDa regions (during the experiment the sizes of the gels were altered, and it caused uncertainty during the size estimation of the proteins).

4.6. Identification of the "ß-lactamase" detected in the 10-14 kDa molecular weight range

The protein was identified from the membrane fraction of strain 19599/3B. The protein detected at 14 kDa was identified by MALDI-TOF as lysozyme, which was used in our experiment to hydrolyse bacterial cell wall. As a control 10 μl of a 1 mg ml^{-1} lysozyme stock solution was applied on 1-D SDS-PAGE, and after regeneration the nitrocefinase activity of lysozyme was detected. We also succeeded in the photometric detection of the nitrocefin- and cefaloridine-hydrolyzing ability of the lysozyme. The enzyme activity for nitrocefin was 0.162 μmol min^{-1}mg lysoszyme^{-1}, for cefaloridine it was 0.0784 μmol min^{-1}mg lysozyme^{-1}. We were not able to measure any hydrolyzing activity for penicillin and oxacillin (antibiotics with penam skeleton), nor for cefamandole (second-generation cephalosporin), and none for cefoperazone (third-generation cephalosporin).

The lysozyme is able to recognize the bacterial cell wall component N-acetylmuramic acid and hydrolyze the glycosidic linkage between N-acetylmuramic acid and N-

acetylglucosamine. It was shown that the structure of penicillin and the positions of its functional groups are highly similar to that of the N-acetylmuramic acid. Probably that is why lysozyme is able to bind to penicillin, but as we also have shown not able to hydrolyze it, and the high concentration of penicillin even inhibits the enzyme. Since the structure of cefaloridine and nitrocefin is somewhat different than the structure of the compounds with penam skeleton, it can provide an explanation for the slow hydrolysis of those molecules by lysozyme.

4.7. Identification of the ß-lactamase detected in the 30-34 kDa molecular weight range

The identification was performed on the extracellular and membrane fractions of all the strains. After 2-D gel electrophoresis nitrocefinase activity could be detected both in acidic (pI 4 – 5) and basic (pI 9 – 10) pH ranges. The molecular weights of proteins at the different pH ranges seemed to differ a little: proteins at the acidic pH range tend to be somewhat larger, but on 2-D gels it does not mean necessarily a real difference. During the analysis of extracellular proteins at least 42% of the significant peaks match with the database peptides of the appropriate protein in case of MALDI-TOF, and at least 5 peptide fragments in case of LC–MS/MS analysis. During membrane protein analysis at least 50% of the significant peaks match with the database peptides of the appropriate protein in case of MALDI-TOF, and at least 3 peptide fragments giving protein score larger than 25 in case of LC–MS/MS analysis.

The identified proteins with acidic or basic pI proved to be almost exclusively the BlaZ penicillinase. In human isolates or in the isolates transferred from human to cow the proteins were identified as BlaZ reference sequence with NCBI-GI number of gi|33416277 (when the manual revision of spectra and peptide fragment lists revealed no possible sequence variant). In strain 7789/47 a BlaZ variant (gi|3603441, called ‘‘type-b’’ ß-lactamase), and in strain 7789/98 its longer variant (gi|67973147) was found together with BlaZ reference sequence (gi|33416277) in extracellular samples or alone in membrane fractions. The database ß-lactamase sequences of the different *Staphylococcus aureus* strains are quite similar, but slight sequence variations are common. These variations can affect the size and possibly the activity of the protein, which can give an explanation for the slow oxacillin-hydrolyzing activity of strain 7789/98 and especially strain 7789/47. In strain a36 and a53 also BlaZ was the only ß-lactamase found. It is possible that the second enzyme was lost or it was masked by another protein, but to be masked it had to be produced in a small amount which is contradictory with its possible importance in borderline phenotype. If it had been lost than in a case of the loss of its plasmid also *blaZ* would have been lost (because strains had only one plasmid). It is much

more plausible that the two bands originally seen on SDS-PAGE renatured gels contained the same protein with different sizes rather than two distinct ß-lactamases. Whatever is true, only BlaZ could be transformed into *Escherichia coli* also by the Italian team.

The different sizes, and perhaps the substrate specificity of the same enzyme could be the consequence of the proteolysis of ß-lactamases or posttranslational modification. A posttranslational modification (mainly phosphorylation) can explain the shift in the pI of the proteins. There are 23 Serine, 13 Threonine, and 13 Tyrosine residues in BlaZ, which can be phosphorylated. When half of them are phosphorylated it can shift the pI from 9.55 to 5 or 4 (result by ProMoST: Protein Modification Screening Tool, University of Wisconsin, Madison, Medical College of Wisconsin, Proteomics Center), and phosphate groups (MW: 95 Da) also can cause an increase in the molecular weight of the protein. The 23 Asparagines and 4 Glutamines can be spontaneously hydrolyzed to Aspartic acid and Glutamic acid, respectively, which also can alter the pI. It is possible, that this modification occurs (or fails to occur, as the enzymes produced after methicillin induction had lower molecular weights) only in strains typable with group V. phages; it also can explain why we measured the lowest oxacillinase activity in the case of strain 7789/47, which was not typable with such phages alone.

Some other proteins with nitrocefinase activity also were identified: α-hemolysin in strain a53, a36, 9800, 9989, and 14287/1; ß- hemolysin in strain 822 and 14287/1; truncated ß-hemolysin in strain 14287/1, γ-hemolysin in strain 9989, and other cytotoxins and virulence factors (leukocidin in strain a53 and a36, SplB serine protease in strain a53 and 14287/1). We probably can say that these proteins hydrolyzed nitrocefin aspecifically and not related to their original function, or it can occur that their presence in those spots had masked the ß-lactamase (though it is not really possible in the case of proteins identified by LC-MS/MS).

5. SUMMARY

BORSA strains were selected based on their oxacillin MIC values out of *Staphylococcus aureus* strains from various sources. All together 7 human and 4 bovine BORSA isolates were found. The 4 bovine and 6 human isolates were *mecA* negative, but strain 822 possessed *mecA*. In this case probably the PBP2a could not function properly.

Most collected BORSA strains were typable with group V. phages and were able to hydrolyse oxacillin. The only bovine isolate which was not typable with those phages was the slowest hydrolyser. The *mecA* carrying strain 822 which was not typable with any phages had the highest oxacillinase activity. This means that carrying group V. phages is not a prerequisite for oxacillin-hydrolysis for this *mecA* bearing strain (contrary to the strains lacking *mecA*).

It was proved that lysozyme used in membrane isolation had antibiotic-hydrolyzing activity. This was showed by spectrophotometric method for nitrocephin and cephaloridine.

It was determined that BlaZ penicillinase is responsible for the oxacillin-hydrolysis. In case of the human isolates and of the two bovine strains with human origin the BlaZ reference sequence with NCBI GI database number gi|33416277 was identified.

The ß-lactamases of the two other bovine isolates were sequence variants of the BlaZ, differing in 7% from the penicillinase of the human isolates. These two strains either had two BlaZ enzymes, or one new, undescribed sequence variant.

In all strains the same enzyme was detected at markedly different pIs (pI 3-5 and pI 9-10). The different pI could be the result of some posttranslational modifications. In silico analysis showed that phosphorylation could be responsible for this. The ~1kDa MW difference could also be the consequence of phosphorylation.

6. PUBLICATIONS

6.1. Publications related to the thesis

Candidate: Judit Keserű

Neptun ID: GSJ6QX

Doctoral School: Doctoral School of Molecular Cell and Immune Biology

List of publications related to the dissertation

1. **Keserű, J.**, Szabó, I., Gál, Z., Massidda, O., Mingoia, M., Kaszanyitzky, J.É., Jánosi, S., Hulvely, J., Csorba, A., Buzás, K., Hunyadi-Gulyás, É., Medzihradszky, K.F., Biró, S.: Identification of beta-lactamases in human and bovine isolates of Staphylococcus aureus strains having borderline resistance to penicillinase-resistant penicillins (PRPs) with proteomic methods.
   Vet. Microbiol. 147 (1-2), 96-102, 2011.
   DOI: http://dx.doi.org/10.1016/j.vetmic.2010.06.006
   IF:3.256 (2010)

2. **Keserű, J.**, Gál, Z., Barabás, G., Benkő, I., Szabó, I.: Investigation of beta-Lactamases in clinical isolates of Staphylococcus aureus for further explanation of borderline methicillin resistance.
   Chemotherapy. 51 (6), 300-304, 2005.
   DOI: http://dx.doi.org/10.1159/000088951
   IF:1.413

6.2. Other publications

List of other publications

3. Birkó, Z., Swiatek, M., Szájli, E., Medzihradszky, K.F., Vijgenboom, E., Penyige, A., **Keserű, J.**, Wezel van, G.P., Biró, S.: Lack of A-factor production induces the expression of nutrient scavenging and stress-related proteins in Streptomyces griseus.
Mol. Cell Proteomics. 8 (10), 2396-2403, 2009.
DOI: http://dx.doi.org/10.1074/mcp.M900194-MCP200
IF:8.791

4. Penyige, A., **Keserű, J.**, Fazakas, F., Schmelczer, I., Szirák, K., Barabás, G., Biró, S.: Analysis and identification of ADP-ribosylated proteins of streptomyces coelicolor M145.
J. Microbiol. 47 (5), 549-556, 2009.
DOI: http://dx.doi.org/10.1007/s12275-009-0032-y
IF:1.463

5. Kaszanyitzky, J.É., Egyed, Z., Jánosi, S., **Keserű, J.**, Gál, Z., Szabó, I., Veres, Z., Somogyi, P.: Staphylococci isolated from animals and food with phenotypically reduced susceptibility to beta-lactamase-resistant beta-lactam antibiotics.
Acta Vet. Hung. 52 (1), 7-17, 2004.
DOI: http://dx.doi.org/10.1556/AVet.52.2004.1.2
IF:0.566

Total IF: 15.489

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The Candidate's publication data submitted to the Publication Database of the University of Debrecen have been validated by Kenezy Life Sciences Library on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

17 January, 2012

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6.3. Lectures and posters related to the thesis

Keserű J, Gál Zs, Hernádi F, Barabás Gy, Szabó I (2001) *Staphylococcus aureus* borderline meticillin rezisztenciájának vizsgálata. A Magyar Kemoterápiai Társaság XVI. Kemoterápiai Konferenciája, Hajdúszoboszló (poster)

Keserű J, Gál Zs, Hernádi F, Barabás Gy, Szabó I (2001) Borderline meticillin rezisztencia mechanizmusának vizsgálata *Staphylococcus aureus* törzseken. A Magyar Mikrobiológiai Társaság 2001. évi Jubileumi Nagygyűlése, Balatonfüred (lecture)

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6.4. Other lectures and posters

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