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Screening for phenotypic and genotypic resistance to antibiotics in Gram positive pathogens

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SUMMARY. Gram positive bacteria such as methicillin resistant *Staphylococcus aureus*, vancomycin resistant enterococci or multidrug resistant *Streptococcus* spp. are increasingly involved in severe infections with serious clinical consequences. The aim of this study is to investigate phenotypic and genotypic resistance traits in Gram positive pathogens isolated from clinical specimens in Cluj-Napoca, Romania. A total number of 31 *Enterococcus* spp., *Staphylococcus* spp. and *Streptococcus* spp. strains were subjected to antimicrobial susceptibility testing by disc diffusion, while the carriage of 26 antibiotic resistance genes and of class 1 integron was assessed by PCR. Bacterial pathogens included in this study were mostly susceptible to folate pathway inhibitors (100%), oxazolidinones (97%), fosfomycins (93%) and glycopeptides (92%). Enterococci, staphylococci and streptococci displayed high levels of phenotypic resistance to penicillins, tetracyclines and macrolides, a percentage of 42% being multidrug resistant. The strains under this study proved to be able to produce β-lactamase enzymes encoded by the *TEM-1* gene and aminoglycoside modifying enzymes due to the carriage of *aac(6')-Ie-aph(2")* gene, to possess ribosomal protection mechanisms for macrolide and tetracycline resistance associated with *ermB*, *ermC* and *tet(M)* genes and to bear efflux genes *tet(A)*, *tet(B)*, *tet(C)* ant *tet(L)*. Class 1 integron integrase was detected in 16% of the isolates, but no significant correlations were found between the carriage of *intI1* gene and the phenotypic or genotypic resistance among the Gram positive pathogens investigated.

Keywords: AMR, MDR, ARG, *Enterococcus* spp., *Staphylococcus* spp., *Streptococcus* spp.

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Introduction

Gram positive bacteria are common causes of severe infections, methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin resistant enterococci (VRE) or multidrug resistant (MDR) *Streptococcus* spp. being of particular concern. The percentage of severe nosocomial infections such as bloodstream infections caused by antimicrobial resistant (AMR) Gram positive bacteria is increasing (Rice, 2006; Attman *et al*., 2015). For instance, penicillin and macrolide resistance is associated with a higher mortality rate in cases of pneumococcal community acquired pneumonia and bacteraemia; infections due to MRSA and VRE are also associated with higher mortality rates, prolonged length of hospital stay, and increased costs (Lode, 2009; Flores-Mireles *et al*., 2015). As a whole, MDR Gram positive pathogens are rapidly becoming an urgent and sometimes unmanageable clinical problem, not only in pneumonology, oncology and urology wards, but also in intensive-care units (Cornaglia, 2009). The serious clinical consequences of AMR among Gram positive pathogens emphasize the importance of efforts to limit their emergence and spread. Susceptibility data can be used by healthcare providers to make rational choices about the use of antimicrobial agents. Furthermore, susceptibility data can guide policy-makers to track and prevent the spread of antimicrobial resistant organisms. Finally, awareness on bacterial resistance traits may stimulate interest in developing new antimicrobial agents and therapies (Lode, 2009).

Previous epidemiological surveys in Romania have focused on the prevalence of Gram positive bacterial infections without molecular screening (Simon *et al*., 2010; Stoian *et al*., 2013; Pîrvănescu *et al*., 2014), which was performed often in Gram negative pathogens (Crăciunaş *et al*., 2010; Flonta *et al*., 2011, Székely *et al*., 2013).

This study investigates antimicrobial susceptibility in Gram positive pathogens isolated from clinical specimens in Cluj-Napoca, Romania. Furthermore, *Enterococcus* spp., *Staphylococcus* spp. and *Streptococcus* spp. isolates displaying phenotypic AMR were screened for multiple mechanisms of genetic resistance. Enzymes involved in antibiotic modification (β-lactamases, aminoglycoside modifying enzymes, alteration of antibiotic target site (peptidoglycan precursors conferring resistance to glycopeptides), protecting proteins (for the DNA- enzyme complexes from the action of quinolones), ribosomal protection mechanisms (for macrolide and tetracycline resistance), efflux proteins (for tetracycline resistance) have been investigated by the PCR screening for 26 antibiotic resistance genes (ARGs). Also, the association of phenotypic and genotypic resistance with the carriage of class 1 integron was assessed in all clinical isolates.

Materials and methods

Bacterial isolates

Clinical specimens of urine and various secretions were collected in a private laboratory from Cluj-Napoca during January-March 2016. After the inoculation of samples into specific culture media including Blood agar, Chapman agar and Bile

Aesculin agar, incubated at 37 °C overnight, presumptive colonies were purified and recultured. The isolates were identified according to standard methods by colony appearance, pigment production, catalase test, oxidase test, Gram staining. Latex agglutination kits (Oxoid) were used for additional serological confirmation.

Antimicrobial susceptibility testing

Enterococcus spp., *Staphylococcus* spp. and *Streptococcus* spp. strains isolated from clinical samples were subjected to antimicrobial susceptibility testing as per the reference guidelines (EUCAST, 2014) against the following antibiotic classes: penicillins, cephems (except for enterococci), glycopeptides, lipopeptides, florquinolones, aminoglycosides, macrolides (except for enterococci), tetracyclines, folate pathway inhibitors (only for staphylococci), oxazolidinones and fosfomycins (only for enterococci). *Enterococcus* spp. were tested for AMR against 9 individual drugs: penicillin, ampicillin, vancomycin, ciprofloxacin, levofloxacin, gentamicin, tetracycline, linezolid and fosfomycin. *Staphylococcus* spp. were tested for AMR against 12 individual drugs: penicillin, cefoxitin, daptomycin, ciprofloxacin, levofloxacin, gentamicin, erythromycin, clarithromycin, tetracycline, trimethoprim-sulfamethoxazole, linezolid and fosfomycin. *Streptoococcus* spp. were tested for AMR against 10 individual drugs: penicillin, ampicillin, cefoxitin, ceftriaxone, cefepime, vancomycin, daptomycin, levofloxacin, erythromycin and linezolid. Antibiotic susceptibility testing was performed by disk diffusion in Mueller-Hinton agar plates (Farkas, 2016). Following overnight incubation, the inhibition zone diameters were measured and the results were interpreted according to CLSI guidelines (CLSI, 2015).

ARG screening

Gram positive pathogens displaying phenotypic resistance to at least one antibiotic were further included in the screening of ARGs conferring resistance to the corresponding antimicrobials. In addition, carriage of class I integron integrase gene was screened in all isolates. PCR amplifications were performed in 25 µl reaction mix containing: 12.5 µl DreamTaq Green PCR master mix (2x), 10.25 µl nuclease-free water, 0.125 µl each primer to a final concentration of 0.5 µM, and 2 µl bacterial suspension adjusted to a 0.5 McFarland standard. The following reaction conditions were set up using a thermocycler TProfessional Trio (Analytik Jena, Germany). After the initial denaturation at 94°C for 5 min, the cycle for denaturation, annealing and synthesis was 35-fold repeated and followed by a final elongation at 72°C for 5 min (Table 1). Amplified PCR products were separated in 1.5% agarose gel in 1 x TBE buffer and stained with ethidium bromide 0.5 µg/ml. Data acquisition and analysis were performed using the BDA Digital Compact System and BioDocAnalyze Software (Analytik Jena, Germany). PCR primers and molecular reagents were purchased from Cleaver Scientific, Eurogentec, Lonza and Thermo Fisher Scientific.

Table 1.

Primers and PCR conditions used in this study

Antibiotic class	Target gene	Primers sequence F/R (5' – 3')	Amplicon size (bp)	Annealing temperature and time
Penicillins	*TEM-1*	GGTCGCCGCATACACTATTC/ ATACGGGAGGGCTTACCATC	500	57°C 45 s
	TEM-2	AAGTAAAAGATGCTGAAGATAAGTTGG/ GATCTGTCTATTTCGTTCATCCATAG	737	61°C 45 s
	SHV-1	GCGTTATATTCGCCTGTGTATTAT/ GCCTGTTATCGCTCATGGTAATG	385	61°C 45 s
Cephems	*AmpC*	AGAAGGACCAGGCACAGATC/ CTCGGCATTGGGATAGTTGC	455	57°C 45 s
Glycopeptides	*vanA*	GCTATTCAGCTGTACT/ CAGCGGCCATCATACGG	781	51°C 45 s
	vanB	CGCCATATTCTCCCCGGATAG/ AAGCCCTCTGCATCCAAGCAC	600	61°C 45 s
Florquinolones	*qnrA*	AGTTTGATGGTTGCCGCTTT/ TCTTCATTGATCTGCACGCC	541	53°C 45 s
	qnrB	TCGTGCGATGCTGAAAGATG/ CCGAATTGGTCAGATCGCAA	368	55°C 45 s
	qnrS	TGATCTCACCTTCACCGCTT/ GAGTTCGGCGTGGCATAAAT	496	55°C 45 s
Aminoglycosides	*aac(3')-I*	ACCTACTCCCAACATCAGCC/ TCTTCCCGTATGCCCAACTT	329	55°C 45 s
	aac(3)-IIIa	GCATGCCTCACTTAAAGCGA/ ACCGTTTCTTCCAAGCATCG	514	55°C 45 s
	aac(6')-Im	GGCTGACAGATGACCGTGTTCTTG/ GTAGATATTGGCATACTACTCTGC	482	61°C 45 s
	aac(6')-Ie-aph(2")	CCAAGAGCAATAAGGGCATA/ CACTATCATAACCACTACCG	400	53°C 45 s
	aac(6')-II	AGCGACCGACTCTTGATGAA/ GGCTTGTCGTGTTTGAACC	414	53°C 45 s
	aph(2)-Ib	CTGAACACAGCAGCGACTAC/ TTGTAATCGCCATGCACCAG	646	55°C 45 s
	ant(4')-Ia	GTCAAAAACTGCTAACACAAG/ AATAATACTGCTAACGATAAT	135	53°C 30 s

Table 1. continued

Macrolides	*ermA*	GAACCAGAAAAACCCTAAAGACAC/ ACAGAGTCTACACTTGGCTTAGGATG	513	61°C 45 s
	ermB	GAAAAGGTACTCAACCAAATA/ AGTAACGGTACTTAAATTGTTTAC	639	51°C 45 s
	ermC	CGTAACTGCCATTGAAATAGACC/ GTGAGCTATTCACTTTAGGTTTAGG	356	61°C 45 s
	mefA	CATCGACGTATTGGGTGCTG/ CCGAAAGCCCCATTATTGCA	516	55°C 45 s
Tetracyclines	*tet(A)*	GCAAGCAGGACCATAATCGG/ GCCGATATCACAGATGGGGA	572	57°C 45 s
	tet(B)	GGTTAGGGGCAAGTTTTGGG/ ATCCCACCACCAGCCAATAA	541	57°C 45 s
	tet(C)	TGAGATCTCGGGAAAAGCGT/ AAAGCCGCGGTAAATAGCAA	460	53°C 45 s
	tet(K)	AGGATCTGCTGCATTCCCTT/ AGCAAACTCATTCCAGAAGCA	822	53°C 45 s
	tet(L)	TATTCAAGGGGCTGGTGCAG/ CGGCAGTACTTAGCTGGTGA	545	57°C 45 s
	tet(M)	CCGTCTGAACTTTGCGGAAA/ CAACGGAAGCGGTGATACAG	627	55°C 45 s
Class 1 integron integrase	*intI1*	CGTGCCGTGATCGAAATCCAG/ TTCGTGCCTTCATCCGTTTCC	371	60°C 45 s

Statistical analysis

Frequencies and proportions of AMR isolates, the abundances of MDR, ARGs and *intI1* bearing strains were calculated. MDR was defined as non-susceptibility to at least one agent in three or more antimicrobial categories (Magiorakos *et al*., 2012).

Inferential statistics by co-dependency was analysed to verifiy whether occurrences of phenotypic and genotypic resistance were correlated with *int1I* carriage. Two-sided 5% significance levels were used to identify statistically significant results. Statistical analyses were performed using the Real Statistics Resource Pack software for Microsoft Excel (Zaiontz, 2015), with a significance level of $p = 0.05$.

Results and discussion

Bacterial isolates

A total number of 31 Gram positive pathogens were isolated in this study and selected for AMR and ARG screening. *Enterococcus* spp. (11 isolates) were isolated from urinary tract infections (nine strains) and from secretions (two strains). *Staphylococcus* spp. (17 isolates) obtained from urocultures (three strains) and from secretions (14 strains) were identified as *S. aureus* (16 isolates) and *S. saprophyticus* (one isolate). The three isolates of *Streptococcus* spp. were obtained from urinary tract infectionss (two strains) and from secretions (one strain), being confirmed as group B *Streptococcus*, group B β-hemolytic *Streptococcus* and group C *Streptococcus*.

Antimicrobial susceptibility

Gram positive pathogens included in this study were mostly susceptible to folate pathway inhibitors (100%), oxazolidinones (97%), fosfomycins (93%) and glycopeptides (92%) (Table 2). *Enterococcus* spp., *Staphylococcus* spp. and *Streptococcus* spp. isolates displayed phenotypic resistance from one to six individual drugs, belonging to up to five antimicrobial categories (Fig. 1). High levels of resistance were observed for penicillins (80%), tetracyclines (72%) and macrolides (58%). A percentage of 42% clinical isolates were multidrug resistant (Fig. 2), MDR etiology being the following: two out of three strains of *Streptococcus* spp., seven out of 17 *Staphylococcus* spp. and four out of ten *Enterococcus* spp. Seven out of 17 *S. aureus* were MRSA while only one out of ten *Enterococcus* spp. were VRE.

Table 2.

Antimicrobial susceptibility of Gram positive isolates

Antibiotic category	Susceptible isolates			
	Enterococci (%)	Staphylococci (%)	Streptococci (%)	Total (%)
Penicillins	5 (45)	15 (88)	0 (0)	6 (19)
Cephalosporins	-	12 (71)	3 (100)	15 (75)
Glycopeptides	13 (93)	-	3 (100)	12 (92)
Lipopeptides	-	8 (50)	1 (33)	11 (58)
Florquinolones	8 (80)	15 (94)	2 (67)	26 (87)
Aminoglycosides	9 (82)	15 (94)	-	25 (89)
Macrolides	-	6 (38)	1 (33)	5 (26)

Table 2. continued

Tetracyclines	1 (9)	4 (57)	-	5 (28)
Folate pathway inhibitors	-	17 (100)	-	17 (100)
Oxazolidinones	10 (91)	5 (100)	3 (100)	28 (97)
Fosfomycins	10 (91)	3 (100)	-	13 (93)

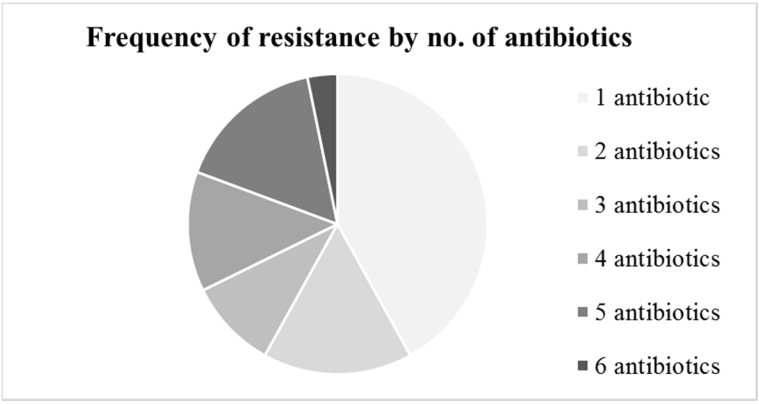

Figure 1. Percentage of strains exhibiting antimicrobial resistance

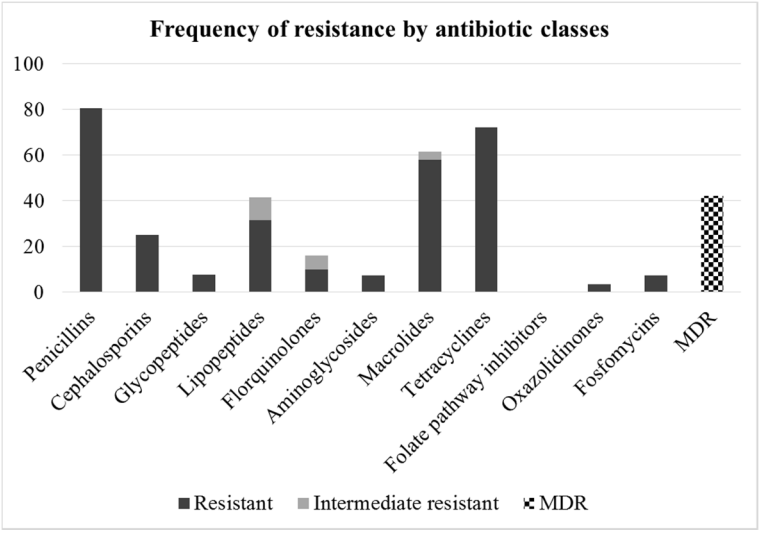

Figure 2. Percentage of resistant, intermediate resistant and MDR strains

ARG screening

Of 25 Gram positive pathogens displaying resistance to penicillins, six (24%) carried the *TEM-1* β-lactamase, while the *TEM-2*, *SHV-1* and *AmpC* genes were not identified in this study. Although *TEM-1* is the most common plasmid-mediated β-lactamase of resistant enteric Gram negative bacilli (Paterson and Bonomo, 2005), this gene was present in four *Enterococcus* spp. isolates and in two *Staphylococcus aureus* strains, one being methicillin resistant (Table 3). *TEM-1* is able to hydrolyze ampicillin at a greater rate than carbenicillin, oxacillin, or cephalothin, and has negligible activity against extended-spectrum cephalosporins. It is inhibited by clavulanic acid (Paterson and Bonomo, 2005).

Aac(6')-Ie-aph(2"), a gene encoding aminoglycoside modifying enzymes was detected in two strains phenotypically resistant to gentamycin (100%), both *Enterococcus* spp. (Table 3). The genes *aac(3')-I*, *aac(3)-IIIa*, *aac(6')-Im*, *aac(6')-II*, *aph(2)-Ib*, *ant(4')-Ia* were not present. The bifunctional *aac(6')-aph(2")* gene, resulted from the fusion of two aminoglycoside modifying enzyme genes, confers high-level resistance to all clinically useful aminoglycosides except streptomycin. The genes responsible for high-level aminoglycoside resistance have in most cases been identified on plasmids and on transposons (Behnood *et al.*, 2013).

Resistance to macrolides was found to be mediated by ribosomal methylation associated with *ermB* gene in *Streptococcus* spp. and with *ermC* gene in MRSA (Table 3), while the *ermA* gene was not present. Methylation of the ribosomal drug binding site, which confers resistance to macrolides, lincosamides, and streptogramin group B (MLSB) may be inducibly or constitutively expressed (Leclercq, 2002). Previously, the most prevalent mechanism of macrolide resistance was also associated with *ermB* gene in *Streptococcus pneumoniae* (Kresken *et al*., 2004; Reinert *et al*., 2008). Drug efflux conferring low-level resistance to erythromycin but not clindamycin, encoded by the *mefA* gene, was not detected in this study.

Tetracycline resistance was found to be encoded by *tet(A)* (8%), *tet(B)* (15%), *tet(C)* (23%), *tet(L)* (23%) and *tet(M)* (69%) (Table 3). Of three *Staphylococcus aureus* isolates resistant to tetracycline, one MRSA strain was positive for both *tet(B)* and *tet(C)* genes. Of ten *Enterococcus* spp. isolates displaying phenotypic resistance, one carried *tet(A)* and *tet(B)* genes, two carried *tet(C)*, three carried *tet(L)* and nine carried *tet(M)* genes. *Tet(K)* was not found among the Gram positive pathogens under this study. Many tetracycline efflux pumps, such as *tet(A)*, *tet(B)* and *tet(C)* genes have been described as specific for Gram negative bacteria. These proteins share similarities with other efflux proteins involved in multiple drug resistance, quaternary ammonium resistance, together with chloramphenicol and quinolone resistance. The *tet(K)* and *tet(L)* genes are generally found on small transmissible plasmids in Gram-positive bacteria (Roberts and Schwartz, 2015). The *tet(L)* gene has often been linked to the trimethoprim resistance gene *dfrK* (Roberts, 2012). The tetracycline ribosomal

protection protein *Tet(M)* confers resistance by catalyzing the release of tetracycline from the ribosomes and also by directly interacting and altering the conformation of the tetracycline binding site. It is tough to be of Gram positive origin, where a mosaic of *tet* genes but also a combination of *tet(M)* and *erm(B)* was identified (Roberts and Schwartz, 2015), being often found in clinical strains of *Enterococcus* spp. and *Staphylococcus* spp. (Schmitz *et al*., 2001; Anderson *et al*., 2017).

The only one VRE isolate was not positive for *vanA* or *vanB* genes. Neither the quinolone resistance mechanisms encoded by *qnrA*, *qnrB* and *qnrS* genes were detected in the three Gram positive pathogens phenotypically resistant to quinolones.

All the Gram positive pathogens were screened for class 1 integron integrase *intI1*, which was found in five out of 31 isolates.

Table 3.

Antimicrobial resistance mechanisms of Gram positive isolates

Antibiotic category	**Genetic mechanism of resistance**	**Prevalence of ARGs**			
		Enterococci (%)	**Staphylococci (%)**	**Streptococci (%)**	**Total (%)**
Penicillins	*TEM-1*	4 (67)	2 (13)	0 (0)	6 (24)
Aminoglycosides	*aac(6')-Ie-aph(2")*	2 (100)	-	-	2 (100)
Macrolides	*ermB*	-	0 (0)	2 (100)	2 (20)
	ermC	-	1 (13)	0 (0)	1 (10)
Tetracyclines	*tet(A)*	1 (10)	0 (0)	-	1 (8)
	tet(B)	1 (10)	1 (33)	-	2 (15)
	tet(C)	2 (20)	1 (33)	-	3 (23)
	tet(L)	3 (30)	0 (0)		3 (23)
	tet(M)	9 (90)	0 (0)	-	9 (70)
Class 1 integron	*intI1*	1 (9)	3 (17)	1 (33)	5 (16)

No significant correlations were found between the carriage of class 1 integron integrase AMR, MDR or ARG detection in Gram positive pathogens isolated from clinical samples under this study.

Conclusions

This study emphasizes the high-level of antimicrobial resistance, multidrug resistance and different mechanisms of genetic resistance among the Gram positive pathogens. *Enterococcus* spp., *Staphylococcus* spp. and *Streptococcus* spp. isolates

displayed high levels of phenotypic resistance to penicillins, tetracyclines and macrolides. A percentage of 42% clinical isolates were MDR strains. Gram positive pathogens are able to produce β-lactamase enzymes encoded by the *TEM-1* gene, aminoglycoside modifying enzymes due to the carriage of *aac(6')-Ie-aph(2")* gene, possess ribosomal protection mechanisms for macrolide and tetracycline resistance associated with *ermB, ermC* and *tet(M)* genes and bear efflux genes *tet(A)*, *tet(B)*, *tet(C)* ant *tet(L)*. No significant correlations were found between the carriage of class 1 integron integrase AMR, MDR or ARG detection in Gram positive pathogens isolated from clinical samples under this study. Class 1 integron integrase gene was detected in 16% of the isolates, but no significant correlations were found between the carriage of *intI1* and the phenotypic or genotypic resistance among the Gram positive pathogens investigated.

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R E F E R E N C E S

Anderson, A.C., Andisha, H., Hellwig, E., Jonas, D., Vach, K., Al-Ahmad, A. (2017) Antibiotic resistance genes and antibiotic susceptibility of oral *Enterococcus faecalis* isolates compared to isolates from hospitalized patients and food, In: *Advances in experimental medicine and biology*, Cohen, I. R., Lajtha, A., Lambris, J. D., Paoletti, R., Rezaei, N. (eds.), Springer, Boston, pp. 1-16

Attman, E., Aittoniemi, J., Sinisalo, M., Vuento, R., Lyytikainen, O., Karki, T., Syrjanen, J., Huttunen, R. (2015) Etiology, clinical course and outcome of healthcare-associated bloodstream infections in patients with hematological malignancies: a retrospective study of 350 patients in a Finnish tertiary care hospital, *Leukemia and Lymphoma* **56**(12), 3370-3377

Behnood, A., Farajnia, S., Moaddab, S.R., Ahdi-Khosroshahi, A., Katayounzadeh, A. (2015) Prevalence of aac (6′)-Ie-aph (2″)-Ia resistance gene and its linkage to Tn5281 in *Enterococcus faecalis* and *Enterococcus faecium* isolates from Tabriz hospitals. *Iranian Journal of Microbiology* **3**, 203-208

CLSI (2015) Performance standards for antimicrobial susceptibility testing; twenty-fifth informational supplement M100-S25, Clinical and Laboratory Standards Institute, Wayne, pp. 238

Crăciunaş, C., Butiuc-Keul, A., Flonta, M., Almaş, A., Brad, A., Sigarteu, M. (2010) Development of a PCR assay for identification of antibiotic resistance determinants at *Staphylococcus aureus*, *Analele Universităţii din Oradea, Fascicula Biologie* **17**(2), 248-252

Cornaglia, G. (2009) Fighting infections due to multidrug-resistant Gram-positive pathogens, *Clinical Microbiology and Infection* **15**(3):209-211

EUCAST (2014) *Antimicrobial susceptibility testing. EUCAST disk diffusion method*. Stockholm: European Committee on Antimicrobial Susceptibility Testing

Farkas, A. (2016) Antibiotic susceptibility testing by disc diffusion method, In: *Methodological guide for monitoring antibiotics and antibiotic resistance in the environment,* Coman, C. (ed.), Accent Publisher, Cluj-Napoca, pp. 253-276

Flonta, M., Lupşe, M., Crăciunaş, C., Almaş, A., Cârstina, D. (2011) Ertapenem resistance among extended-spectrum-β-lactamase producing *Klebsiella pneumoniae* isolates, *Therapeutica* **15**(2), 121-125

Flores-Mireles, A.L., Walker, J.N., Caparon, M., Hultgren, S.J. (2015) Urinary tract infections: epidemiology, mechanisms of infection and treatment options, *Nature Reviews Microbiology* **13**, 1-16

Kresken, M., Henrichfreise, B., Bagel, S., Brauers, J., Wiedemann, B. (2004) High Prevalence of the *ermB* gene among erythromycin-resistant *Streptococcus pneumoniae* isolates in Germany during the winter of 2000-2001 and in vitro activity of telithromycin, *Antimicrobial Agents and Chemotherapy* **48**(8), 3193-3195

Leclercq, R. (2002) Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications, *Clinical Infectious Diseases* **34**,482–492

Lode, H. M. (2009) Clinical impact of antibiotic-resistant Gram-positive pathogens. *Clinical Microbiology and Infection* **15**(3):212-217

Magiorakos, A.P., Srinivasan, A., Carey, R.B., Carmeli, Y., Falagas, M.E., Giske, C.G., Harbarth, S., Hindler, J.F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D.L., Rice, L.B., Stelling, J., Struelens, M.J., Vatopoulos, A.,Weber, J.T., Monnet, D.L. (2012) Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance, *Clinical Microbiology and Infection* **18**, 268–281

Paterson, D.L., Bonomo, R.A. (2005) Extended-spectrum β-lactamases: a clinical update, *Clinical Microbiology Reviews* **18**, 657-686

Pîrvănescu, H., Bălăşoiu, M., Ciurea, M.E., Bălăşoiu, A.T., Mănescu, M. (2014) Wound infections with multi-drug resistant bacteria, *Chirurgia* **109**, 73-79

Reinert, R.R., Filimonova, O.Y., Al-Lahham, A., Grudinina, S.A., Ilina, E.N., Weigel, L.M., Sidorenko, S.V. (2008) Mechanisms of macrolide resistance among *Streptococcus pneumoniae* isolates from Russia, *Antimicrobial Agents and Chemotherapy* **52**(6), 2260-2262

Rice, L.B. (2006) Antimicrobial resistance in Gram-positive bacteria, *American Journal of Medicine* **119**(6), S11-19

Roberts, M.C. (2012) Mechanisms of bacterial antibiotic resistance and lessons learned from environmental tetracycline resistant bacteria, In: *Antibiotic resistance in the environment,* Keen, P., Montforts, M. (eds.), John Wiley & Sons, Hoboken, pp. 93–121.

Roberts, M.C., Schwarz, S. (2015) Tetracycline and phenicol resistance genes and mechanisms: importance for agriculture, the environment, and humans, *Journal of Environmental Quality* **45**(2), 576-592

Simon, L.M., Junie, L.M., Homodorean, D. (2010) Antimicrobial resistance of staphylococcal strains isolated from various pathological products. *Applied Medical Informatics* **27**(4), 74-80

Schmitz, F.J., Krey, A., Sadurski, R., Verhoef, J., Milatovic, D., Fluit, A.C. (2001) Resistance to tetracycline and distribution of tetracycline resistance genes in European *Staphylococcus aureus* isolates, *Journal of Antimicrobial Chemotherapy* **47**(2), 239–240

Stoian, A.C., Dumitrescu, F., Niculescu, I., Iocu, C., Marinescu, L., Stănescu, M., Stoian, G.R. (2013) Gram positive bacterial infections in immunocompromised patients with HIV infection, *BMC Infectious Diseases* **13**(S1), P30

Székely, E., Damjanova, I., Jánvári, L., Vas, K.E., Molnár, S., Bilca, D.V., Lőrinczi, L.K., Tóth, A. (2013) First description of bla NDM-1, bla OXA-48, bla OXA-181 producing *Enterobacteriaceae* strains in Romania, *International Journal of Medical Microbiology* **303**(8), 697-700

Zaiontz, C. (2015) Real Statistics Using Excel, www.real-statistics.com