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Original Contribution

IN VITRO STUDY OF THE RESISTANCE OF PROBLEMATIC FOR HOSPITAL INFECTIOUS PATHOLOGY MICROORGANISMS TO ANTIMICROBIAL DRUGS

E. Savov*, E. Kjoseva, N. Borisova, I. Gergova, G. Ronkova, A. Trifonova

Department of Microbiology, Military Medical Academy (MMA) , Sofia, Bulgaria

ABSTRACT

Multiresistant bacteria are significant problem for the hospital infectious pathology and the antimicrobial resistance can result in increases morbidity, disease and economic burden and mortality. This study analyzed the data, obtained as a part of monitoring of the resistance of some problematic for hospital pathology bacteria – Esherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii, Staphylococcus aureus, Enterococcus faecalis for 2008 at MMA. There was registered a significant increase in the proportion of multiresistant bacteria to the main groups of antimicrobials used in the clinical practice – third-generation of cephalosporins, carbapenems, quinolones and aminoglycosides. Also, we make an attempt to recognize and explain some of the resistance mechanisms in E.coli, K.pneumoniae and A.baumannii. By this way, the surveillance of antimicrobial resistant portions provides data that are needed to raise the awareness to the problem and instigate necessary optimal interventions.

Key words: multiresistant bacteria, ESBL, MRSA

INTRODUCTION

The analysis of the etiological structure and resistance of microorganisms to antimicrobial drugs in a multiprofile hospital gives considerable information on beginning and spread of microorganisms which are the problem for the hospital pathology, and it defines to great extend the behavior and the politics in the usage of one or another antibiotic in the clinical practice of the hospital. Hospitals world-wide are faced with increasingly rapid emergence and spread of resistant bacteria (1). Both antibacterial resistant Gram-negative bacilli and Gram-positive bacteria are reported as important causes of hospital-acquired infections. In many cases, few antibacterials can use for effective treatment, especially with respect to methicillin-resistant Staphylococcus aureus /MRSA/, vancomycin-resistant S.aureus and Enterococcus spp and Gram-negative bacteria, producing extended-spectrum beta-lactamase /ESBL/ which is significant problem for clinical practice.

The aim of this study is to investigate the resistance of some problematic for hospital infectious pathology bacteria to antimicrobial drugs for 2008 with respect optimization of antimicrobial therapy in patients with severe bacterial infections.

MATERIALS AND METHODS

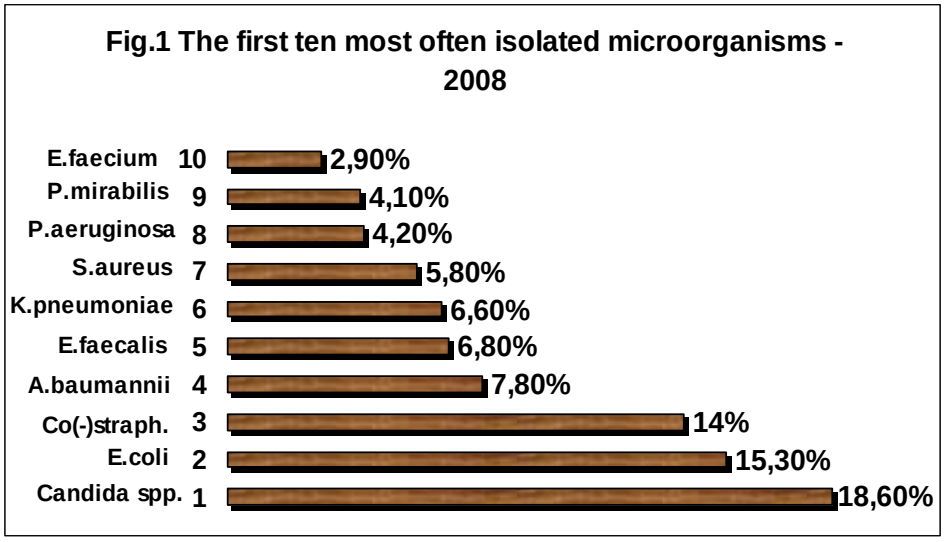
Military Medical Academy /MMA/ in Sofia, Bulgaria is a community hospital with 800 beds. The hospital is a one of the national centres for trauma, respiratory disease, liver transplantation patients' treatment. Antibiotic stewardship at the Military Medical Academy /MMA/ includes all groups of antibiotics together with carbapenems, quionolones, third and forth generations of cephalosporins. Because of that, the MMA is a multiprofile hospital /with several surgery units, two ICU/ it can be a pattern for the tendency in the bacterial resistance' development to antimicrobial drugs in Bulgaria, nevertheless

***Correspondence to:** *E.Savov, Military Medical Academy, 3, G.Sofiiski, Sofia 1606, Bulgaria, E-mail: savove@yahoo.com*

the variety, detected in different regions in the world, countries, hospital to hospital in the same country, reported in this respect. 13565 samples were investigated in the Laboratory of microbiology during 2008. The resistance of the isolates to antimicrobial drugs was done by automated system VITEC TWO v. 4.1. /Biomerieux/ and disk-diffusion method of Bauer, A. et al (2) according to the recommendations of CLS I 2007 (3).

RESULTS AND DISCUSSION

In the laboratory of microbiology were isolated 6826 microorganisms /duplicate were excluded/ during 2008. The results received show that 48,9% of all isolated bacteria are Gram-negative microorganisms. The data show in the analyze of the causative agents structure by species, **(Fig. 1)** that the yeasts with medical significance (*Candida spp* , etc) take first place, nevertheless that in 63%/ predominantly isolated from reproductive system infections and respiratory tract infections/ they were probably estimated as colonizing these anatomical parts of infection. But, it is necessary to note that 27% from Candida spp were found in urine were isolated in significant value. It impresses also the increasing of the relative part of Enterococcus faecium which is in the scale of the first ten most often isolated microorganisms for 2008 **(Fig. 1)**.

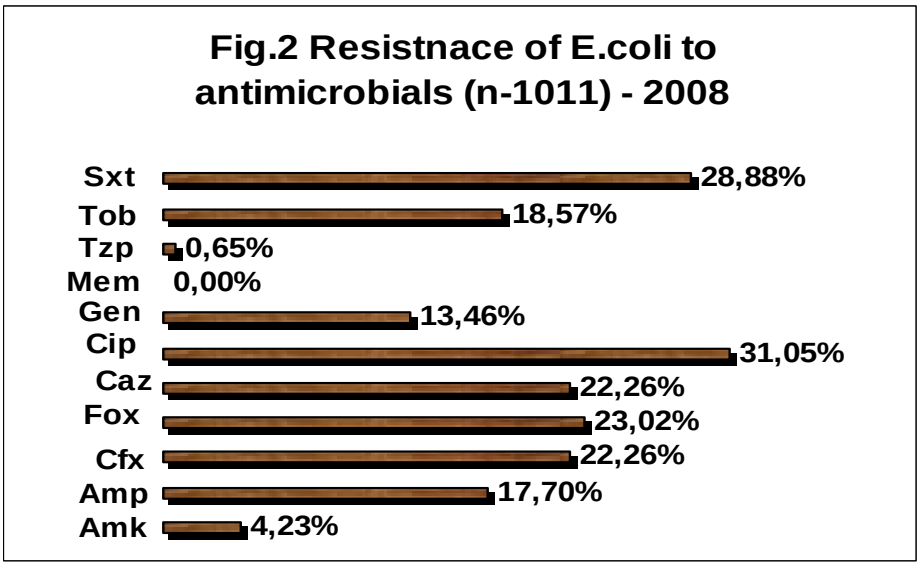


The evaluation of the resistance of some microorganisms to antimicrobial agents is based on the high relative portion which these microorganisms take in the structure of the bacterial infections and problems, concerning treatment of the infections, caused by them.

1. Resistance of E.coli strains to antimicrobial agents

E.coli is the most frequent bacteria causing nosocomial infections. The increasing of the resistance of these microorganisms to antimicrobial drugs during the last years is connected probably with the production of extended-spectrum beta-lactamases /ESBL/. The relative part of the strains, producing ESBL in the units of Military Medical Academy in the last 3 years is about 20% and the most high portion of these strains were isolated from the Anesthesiology and Resuscitation Clinic 37.7%, Bile-liver surgery unit 11.3% and Intensive care unit /ICU/ 8.3%. Very important issue in this respect is also the high part of the E.coli strains /7.8%/ producing ESBL, isolated in outpatients, as well as the ceftazidim' resistance increasing from 13% for 2005 up to 22.2% for 2008. The resistance for meropenem is in the frame 0.0-0.6%. **(Fig. 2)**. In a huge study in Bulgaria Markovska, R. et al / 4 / represents data, concerning the first strains E.coli in Bulgaria, isolated in clinical samples in 1999, producing SHV 12 and also the strains, producing CTX-M-3 and CTX-M-15 ESBL, isolated in 2001 and 2002. Ertapenem resistance can be explain by both outer membrane defect or by CTX-M-2 ESBL production (5). The resistance to beta-lactams in E.coli strains could be also associated with plasmid mediated AmpC /PMAC/ beta-lactamases, such as CMY-type or ACC-type

enzymes, which confer resistance to extended-spectrum cephalosporins. Fortunately, this type of resistance is very low 0.21-0.28% in France (6). Resistance of E.coli strains to fluoroquinolones has consistently increased after 2005. According to EARSS data for 2007 (7) the level of the resistance to fluoroquinolones increased from 5% in 2005 up to 30-53% in some countries in Europe – Malta 35%, Cyprus 40%, Turkey 53%. Cataneo,C. et al (8) reported the fluoroquinolones' resistance of 86.7% in E.coli strains, isolated in patients with haematological malignancies in Italy. For Military Medical Academy in Sofia the resistance to ciprofloxacin is about 30% **(Fig. 2).** The resistance to aminoglycosides is between 13.5% and 18.5% and it is very closed to this for the country /about 20%/ according EARSS data (7)**.**



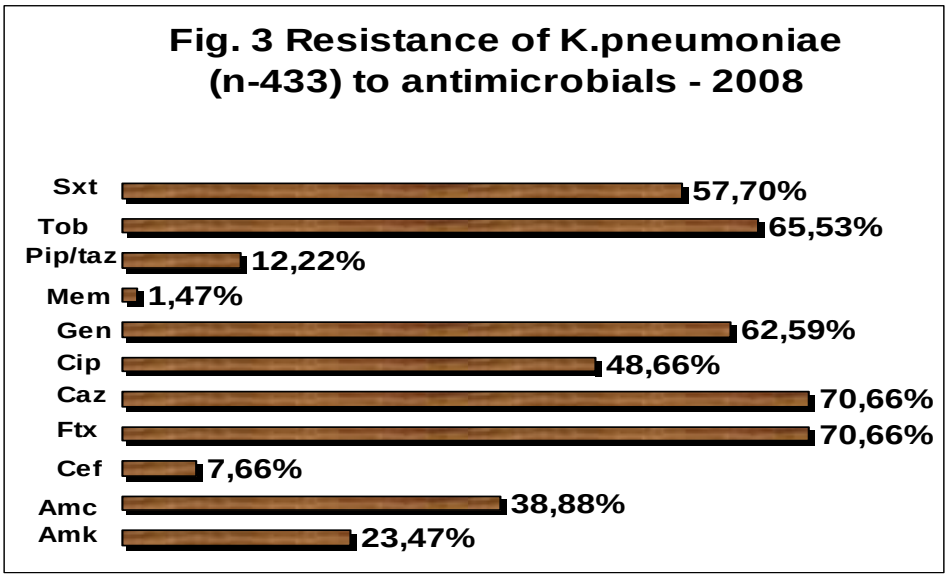
Sxt–sulfam/trimet ,**Tob**-tobramycin ,**Tzp–**tazobactam, **Mem** – meropenem, **Gen**–gentamicin,**Cip**–ciprofloxacin,**Caz**–ceftazidim,**Fox–**cefoxitin,**Cfx**-cefotaxim, **Amp**-ampicillin,**Amk**-amikacin

2. Resistance of Klebsiella pneumoniae strains to antimicrobial drugs

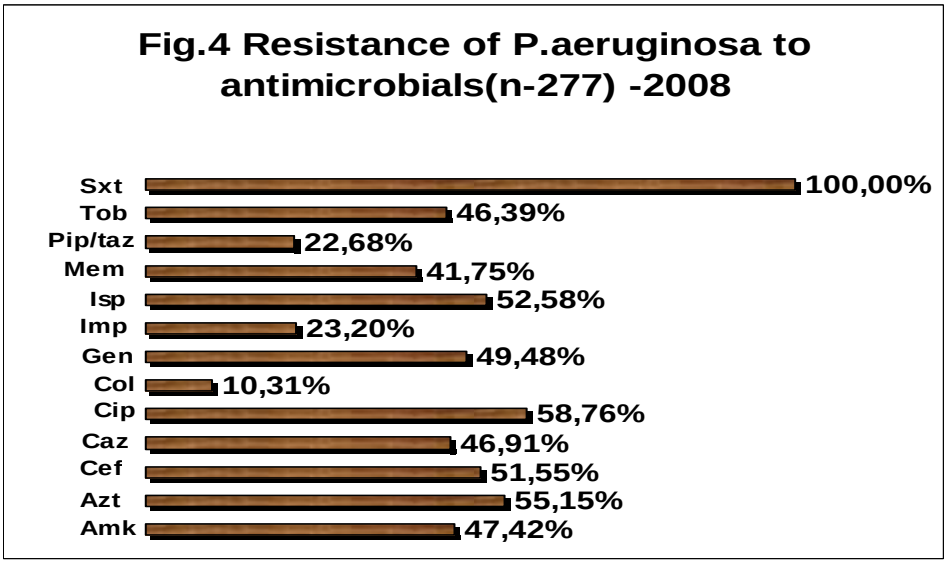
In comparing with 2007, the relative part of K.pneumoniae strains, producing ESBL significantly increased from 53% in 2007 up to 67.8% in 2008, which is in a good correlation with ceftazidim resistance – 70.1%, registered in 2008 /Fig. 3/. Markovska,R. et al (4) reported for the first K.pneumoniae strains in Bulgaria, isolated in 1999, producing SHV2 and SHV5 ESBL and also for strains with CTX-M-15 and CTX-M 3 ESBL isolated during 2001 and 2002. The strains with the highest percentage of ESBL originated from the Anesthesiology and Resuscitation Clinic 55.2%, ICU 12% and Bile-liver surgery unit – 10.1%. Resistance to quinolones is relatively high – 48.7% and to aminoglycosides is 23.5% for amikacin and 62.6% for gentamicin. **(Fig.3)**.

3. Resistance of Pseudomonas aeruginosa strains to antimicrobial drugs

P. aeruginosa is intrinsically resistant to the majority of antimicrobial compounds due to its selective ability to exclude various molecules from penetrating its outer membrane. It is a problem, because in many cases the P.aeruginosa strains isolated were multiresistant with resistance to piperacillin/tazobactam 22.7%, cefepim 51.5% and ceftazidim – 46.9%. The level of the resistance to carbapenems is about 32.2% for imipenem and 41.7% for meropenem as well **(Fig. 4).** The spread of similar multiresistant strains is very important for big hospital complexes and according to Edalucci,E., et al (9) this multiresistance usually is connected with production of metallo-beta-lactamase /MBL/ VIM-2 and also these widespread clones, responsible for human infections, belong to O11 and O12 serotypes. Resistance to ciprofloxacin and aminoglycosides is also high - 58.8% and 47-49% respectively **(Fig. 4)**. Very similar results for increasing of the relative part of multiresistant P.aeruginosa strains for the period 2003-2005 reported also Kirikae,T., et al (10).



Sxt–sulfam/trimet ,**Tob**-tobramycin **Pip/taz**–piperacilintazobactam, **Mem** – meropenem, **Gen**–gentamicin,**Cip**–ciprofloxacin, **Caz** –ceftazidim,**Ftx**-cefotaxim,**Cef**-cefepim, **Amc-**amoxicillin/clav.acid,**Amk**-amikacin

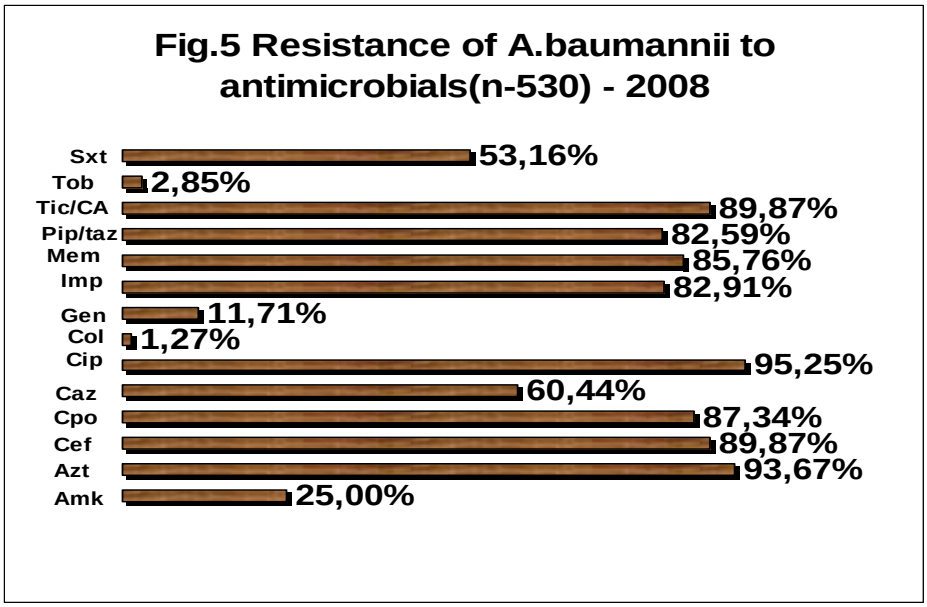


Sxt–sulfam/trimet ,**Tob**-tobramycin ,**Pip/taz**–piperacilin/tazobactam, **Mem** – meropenem, **Isp**–isepamycin,**Imp**-imipenem,**Gen**–gentamicin,**Col**-colistin,**Cip–**ciprofloxacin,**Caz**–ceftazidim,**Cef-**cefepim,**Azt-**aztreonam,**Amk**-amikacin

4. Resistance of Acinetobacter baumannii strains to antimicrobial agents

The analysis of the frequency of the microorganisms isolated in the units at MMA with high risk of infection, confirms the high portion of infections, caused by A.baumannii – for Anesthesiology and Resuscitation Clinic 19.6%, ICU 19.2%, Urology 10.1%, Bile-liver surgery unit 11.7%, Neurosyrgery 12.3%, Abdominal surgery 8.0%. The portion of A.baumannii strains isolated from haemocultures is 8.3%. The resistance of A,baumannii strains to different groups of antimicrobials is saved very high during last 3 years – to ciprofloxacin 95.2%, pipracillin/tazobactam 82.6%, to carbapenems 82.9% for imipenem and 85.8% for meropenem. **(Fig. 5)**. The resistance to carbapenems at MMA is associated with the production of Oxa 23 and Oxa 58 carbapemenases, but do not to metallo-beta-lactamases, in the strains, isolated

predominantly in ICU and Anesthesiology and Resuscitation Clinic. (11). By this way is confirmed the wide geographic spread of the bla oxa23 and bla oxa58 genes in A.baumannii strains but also their appearance in epidemic strains (12) . According to Lee,H., et al (13) in respiratory tract infections in more cases form biofilm these multiresistant A.baumannii strains which possess bla per1 genes, encoding PER 1 ESBL. That's why, the international network for study and prevention of the antimicrobial resistance development /INSPEAR/ during 2001 determinates the appearance of carbapenem resistant Acinetobacter strains as a global warning event, which needs special microbiological and epidemiological measures (14).



Sxt–sulfam/trimet ,**Tob**-tobramycin, **Pip/taz**–piperacilin/tazobactam, **Mem** – meropenem, **Imp**-imipenem**,Gen–**gentamicin**,Col**-colistin**,Cip**–ciprofloxacin,**Caz**–ceftazidim,**Cef-**cefepim,**Azt-**aztreonam,**Amk**-amikacin,**Cpo**-cefpirom

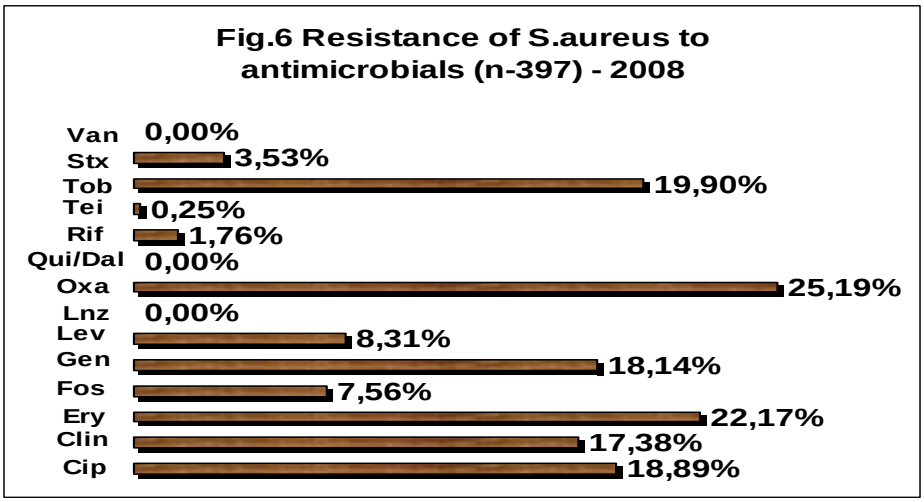
5. Resistance of Staphylococcus aureus strains to antimicrobial drugs

The staphylococci is an important cause of nosocomial disease worldwide. According to our data for 2008 the staphylococci put together about 20% in the etiological structure of nosocomial infections at the MMA. The portion of S.aureus is 6% /Fig.1/ and relative part of MRSA is 25% /Fig.6/., the value which is very similar to the data received from all Mediterranean countries, Romania, the United Kungdom and Irland. (7). There is not resistance, registered to vancomycin, teicoplanin, linezolid and quinopristin/dalfopristin. The resistance to levofloxacin is saved in low level – 8%. (**Fig. 6)**.

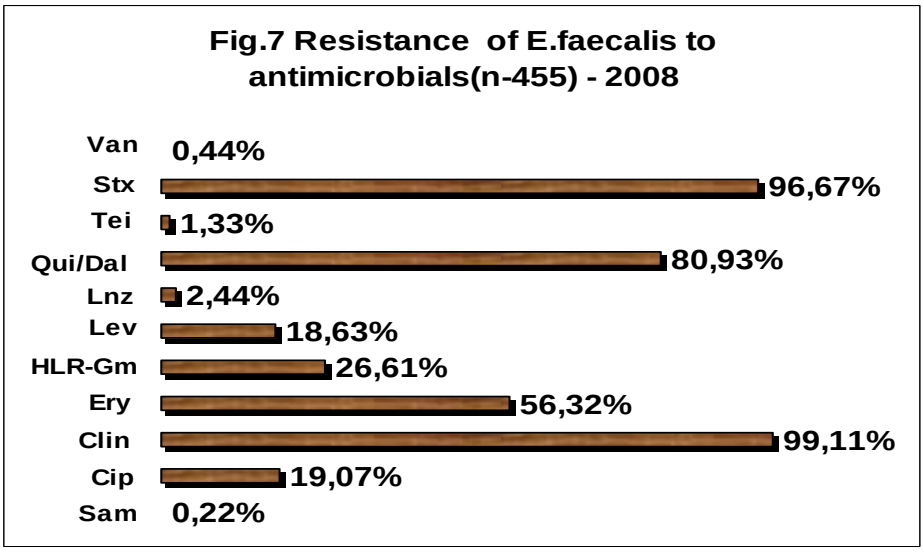
6. Resistance of Enterococcus faecalis strains to antimicrobial drugs

Enterococci are intrinsically resistant to a broad range of antibiotics including cephalosporins, penicillins, sulphonamides and low concentration of aminoglycosides. / EARSS 2007/. As a possibility for treatment of infections, caused by E.faecalis stay vancomycin with 0.4% resistance for 2008, teicoplanin 1.3%, linezolid 2.4%, combination ampicilin/sulbactam 0.22%. **(Fig. 7)**. Patient safety in hospitals is challenged by the ability of enterococci to acquire additional resistance through the transfer of plasmids and transposons and recombination or mutation. The emergence of E.faecalis and E.faecium was paralleled by the increases in glycopeptides and high-level aminoglycoside resistance. Our data show high-level resistance to gentamicin 26.6% for E.faecalis and 83% for E.faecium. These results are in a good correlation with EARSS data for Iceland 13%, Germany 67%. (7). Epidemiological data collected over the last two decades have documented the emergence of enterococci, and in particular E.faecium, as important nosocomial pathogens, which it seen as the expansion of major hospital adapted clonal

lineage /CC17/ (7, 15). This clonal spread together with the outbreaks of vancomycin resistant E.faecium occurs on the background of high-level aminoglycoside resistance. The control of glycopeptides resistant enterococci remains a formidable task for hospital infection control practioners and it is not difficult to predict that these problematic pathogens will continue to remain a challenge in the big hospital complexes.



Van-vancomycin,**Stx**-sulfam/trimet,**Tob**-tobramycin,**Tei**-teicoplanin,**Rif**-rifamycin,**Qui/Dal**-quinopristin/dalfopristin,**Oxa**-oxaccillin,**Lnz**-linezolid,**Lev**-levofloxacin,**Gen**-gentamicin,**Fos**-fosfomycin,**Ery**-erytromycin,**Clin**-clindamycin,**Cip**-ciprofloxacin



Van-vancomycin,**Stx**-sulfam/trimet,**Tei**-teicoplanin,**Qui/Dal**-quinopristin/dalfopristin,**Lnz**-linezolid,**Lev**-levofloxacin,**Ery**-erytromycin,**Clin**-clindamycin,**Cip**-ciprofloxacin,**Sam**-ampicillin/sulbactam, **HLR-Gm**-high level resistance to gentamicin

CONCLUSION

The success in the medicine, in particular in the infectious diseases' control is very close connected with optimal usage of antimicrobials in the clinical practice. Despite of enormous work and the definitive success achieved in the infections treatment, evolutionary developed ability for adaptation of the microorganisms, produces new tasks before the specialists. In this sense, the monitoring of bacterial resistance to antimicrobial drugs represents may be the most important measure of bacterial infections treatment in big hospitals. Obviously, the local pattern of resistance determinates in significant level the choice of empiric therapy, especially in the cases, when

it concerns hospital, multiresistant strains. In contrary, the absence of optimum in antimicrobial therapy supposed not only her failure and disgrace, but unnecessary economic burden because of prolonged hospital stay and the high prices the antimicrobials used.

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