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Frequency of isolation and antibiotic resistance patterns of bacterial isolates from wound infections

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***Abstract*:**

Stojanović-Radić, Z., Dimitrijević, M., Stanković, N., Aleksić, A., Pejčić, M.: ***Frequency of isolation and antibiotic resistance patterns of bacterial isolates from wound infections. Biologica Nyssana, 7 (2), December 2016: 151-158.***

Six hundred and thirteen bacterial strains were isolated from wound swabs and the isolates were identified on the basis of growth on differential and selective media. In order to test the sensitivity of isolated strains to different antibiotics, the disc diffusion method, according to EUCAST protocol v 5.0 was used. The most common species isolated from wound swabs was *Staphylococcus epidermidis* (18.4%), followed by *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Enterococcus faecalis* (16.8%, 12.7% and 10.4%, respectively). The maximum resistance of Gram-positive cocci was observed to penicillin and the lowest to linezolid. Gram-negative bacteria showed the highest resistance to tetracyclines, while the same strains demonstrated the highest sensitivity to polypeptide antibiotics. Comparison of the resistance patterns of Gram-negative and Gram-positive bacterial strains showed significant difference in the tetracycline efficiency.

Key words: skin infections, wound swab, antibiotic resistance, *S. epidermidis*, *S. aureus*, *P. aeruginosa*

***Apstrakt*:**

Stojanović-Radić, Z., Dimitrijević, M., Stanković, N., Aleksić, A., Pejčić, M.: ***Učestalost i rezistencija bakterijskih izolata iz infekcija rana. Biologica Nyssana, 7 (2), Decembar 2016: 151-158.***

Mikrobiološkom analizom uzoraka, izolovano je 613 bakterijskih sojeva iz briseva rana. Izolati su identifikovani na osnovu rasta na diferencijalnim i selektivnim podlogama. Ispitivanje osetljivosti izolovanih sojeva na različite antibiotike rađeno je metodom disk difuzije po EUCAST v 5.0 protokolu. Najčešće izolovana bakterija iz briseva rana bila je *Staphylococcus epidermidis* (18.4%), nakon koje su najučestalije izolovane bile *Staphylococcus aureus*, *Pseudomonas aeruginosa* i *Enterococcus faecalis*. Najveća rezistencija Gram-pozitivnih koka primećena je na peniciline a najmanja na linezolid. Gram-negativne bakterije su pokazale najveću rezistenciju na tetracikline, dok su isti sojevi ispoljili najveću osetljivost na antibiotike iz klase polipeptidnih antibiotika. Poređenjem obrazaca rezistencije Gram-negativnih i Gram-pozitivnih bakterijskih sojeva utvrđena je značajna razlika u efikasnosti primene tetraciklina.

Key words: infekcije kože, bris rane, antibiotska rezistencija, *S. epidermidis*, *S. aureus*, *P. aeruginosa*

Introduction

Loss of skin integrity (trauma) caused by mechanical, biological or chemical agent provides a suitable environment for infectious agent entrance, colonization and consequenting acute/chronic infection (Giacometti et al., 2000; Macedo & Santos, 2005). Wound infections are very frequent in outpatients and clinical patients, where they constitute a major healing barrier, which affects patient's quality of life. Such infected wounds are the cause of large patient's physical and mental discomfort owing to associated pain, hypersensitivity and accompanying unpleasant odour (Kotz et al., 2009). The most prevalent organisms that have been associated with acute and chronic wound infections are *Staphylococcus aureus* (including MRSA – methycillin resistant *Staphylococcus aureus*), *Pseudomonas aeruginosa* and members of family Enterobacteriaceae, such as *Escherichia coli*, *Proteus vulgaris*, *Klebsiella sp.*, yeast *Candida albicans* and others (Akinkunmi et al., 2014; Bessa et al., 2015).

Emerging occurence and spread of (multi)drug resistant microbial pathogens present a significant challenge in providing an effective health care and, therefore, a worldwide medical issue. Extensive use of antimicrobial agents in the treatment of commonly isolated pathogens results with emergence of their increased resistance to these drugs. Also, prescribing of broad spectrum antibiotics for uncomplicated infections presents one of the most responsible approaches for emerging of resistance (McQuiston Haslund et al., 2013). Among resistant microbes, those showing multiresistance (MDR – multiple drug resistance) are the main concern in antimicrobial treatment today, owing to accompanying morbidity, mortality and costs (Veličković-Radovanović et al., 2009). Among isolates from wound infections, many of them represent multiresistant microorganisms. Literature on this subject showed alarming data, with reported multiresistance of many bacterial species, such as *P. aeruginosa*, *S. aureus* (MRSA), as well as species from genera *Enterococcus* and *Acinetobacter* (Cetinkaya et al., 2000; Rice, 2001; Sader et al., 2001; Guzman-Blanco et al., 2009; Moniri et al., 2009). Due to the fact that there are differences in recommendations considering the empiric first-choice antibiotic treatment among European countries (McQuiston Haslund et al., 2013), diverse reports on isolation frequency and resistance patterns for the same species, but isolated in different countries, are not a surprising fact (Sader et al., 2001; Shittu et al., 2002; Hiransuthikul et al., 2004; Oguntibeju & Nwobu, 2004; Macedo & Santos, 2005; Anguzu & Olila, 2007; Pondei et al., 2013). In Serbia, study in Aleksinac (Stanković-Nedeljković et al., 2012) investigated frequency of *P. aeruginosa* isolates from wound infection swabs and reported its high incidence rate of 36.6%. Jovanović et al. (2014) investigated isolation frequencies of surgical and traumatic wounds causative agents and confirmed that there are differences in incidences among different Clinics (Abdominal surgery Clinic and Orthopedy and Traumatology Clinic in Zemun and Belgrade). Among isolated bacteria, the most prevalent ones were *E. coli* (48%), *Pseudomonas spp.* (12%), *Klebsiella spp.* (11%), *Acinetobacter spp.* (7%) in abdominal wounds, while *S. aureus* (36.4%), *Pseudomonas spp.* (20.1%) and *Acinetobacter spp.* (7.1%) were the most common causative agents of traumatic wound infections.

According to the presented facts, periodical surveillance of predominant pathogens and also reporting of their antimicrobial resistance patterns are neccessary actions in each country and its distant regions, whose results will be applied in adjusting therapies to the application of proper and effective antibiotics. Therefore, the goal of this study was to investigate local (territory of Niš) predominant pathogens, recovered from wound infections during a one year period (January to December, 2015). Also, the study included determination of their resistance patterns in order to, based on these results, recommend the best antimicrobial therapy approach for the treatment of wound infections in Niš region.

Material and methods

Specimen collection

The present study has been conducted by analyzing 526 wound swab samples, collected from wound infections of polyclinic Human (Niš) outpatients. The wound samples were collected using a sterile cotton swab, which were used for gentle swabbing of the inner surface of the infected area, afterwards the swabs were aseptically transported to the laboratory. Bacterial samples were collected during a one year period between January and December 2015. A total of 613 bacterial isolates were covered by analysis in order to determine the frequency and resistance patterns of bacterial strains.

Identification of the pathogenic strains

Isolation of bacteria from wound swabs was done by inoculating the samples on blood agar. The inoculated media were incubated at 35-37 ºC for 16 to 24 hours. Bacterial colonies on the agar plates were transported to a panel of selective and differential

media (HiMedia, India) in order to complete their identification to the genus or species level, afterwards bacterial isolates were further identified using a battery of biochemical tests. Endo and MacConkey agar were used for identification of the strains belonging to the family Enterobacteriaceae. Morphological and cultural characteristics were observed on Kligler's double sugar medium and peptone water to demonstrate the indole production. Other biochemical tests included citrate and urea utilization, and also fermentation of mannitol according to Bergey's Manual of Determinative Microbiology (2000).

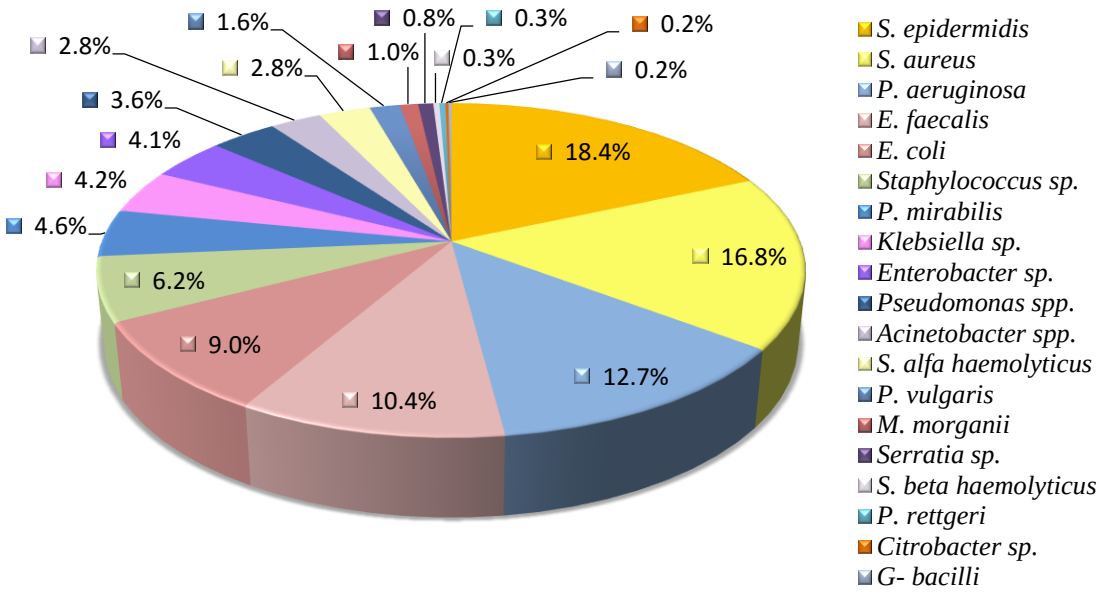

Fig. 1. The frequency of bacteria isolated from wound swab

Susceptibility of isolates to antibiotics

Susceptibility testing was performed according to the standardized EUCAST protocol v 5.0. Briefly, overnight cultures of isolates on Mueller Hinton agar incubated at 37 °C for 24 h were used for making bacterial suspensions in 0.85% NaCl (w/w) and adjusted to McFarland 0.5 turbidity standards. Prepared suspensions were used for inoculation of the Mueller Hinton agar plate's surface, afterwards the antibiotic discs were placed on the inoculated agar surface. After the incubation period of 24 h at 37 °C, the inhibition zones around the discs were measured and interpretation of the inhibition zone values (S-sensitive / R - resistant) was based on the EUCAST v 5.0 criteria.

Results and discussion

The six hundred and thirteen strains were obtained from 526 samples of wound swabs collected during 2015. The most frequently isolated species was *S. epidermidis* (18.4%), which belongs to the Gram-positive cocci. Two species of Gram positive cocci, *S. aureus* and *E. faecalis* were also isolated with high frequency, 16.8% and 10.4% respectively. The most common Gram-negative bacteria in samples were *P. aeruginosa* (12.7%) and *E. coli* (9.0%). The other bacteria were isolated in a relatively low percentage, and *Citrobacter* sp. was the least detected isolate in the swabs (**Fig. 1**).

The results of our research indicate no correlation with previous studies on this subject, conducted in other countries, where *S. epidermidis*, reported here as the most dominant isolate showed relatively low isolation frequency (Sader et al., 2001; Shittu et al., 2002; Hiransuthikul et al., 2004; Oguntibeju & Nwobu, 2004; Macedo & Santos, 2005; Anguzu & Olila, 2007; Pondei et al., 2013). Two different studies on isolation frequency of wound isolates in Brasil (Sader et al., 2001; Macedo & Santos, 2005) showed *S. aureus* and *P. aeruginosa* to be the dominant ones. Investigation in Thailand after tzunami (Hiransuthikal et al., 2004) reported *Aeromonas sp.* to be the most common wound pathogen, followed by *E. coli* and *S. aureus*. Similar research in Uganda (Anguzu & Olila, 2007) showed that *P. mirabilis* ranks as the second one by its isolation frequency, right after *S. aureus*, which was the most frequent wound pathogen. In Nigeria, investigation of Shittu et al. (2002) reported *S. aureus* (25.3%), *E. coli* (12.3%) and *P. aeruginosa* (9.3%) to be the most prevalent isolates from wounds. Two more recent studies (Oguntibeju & Nwobu, 2004;

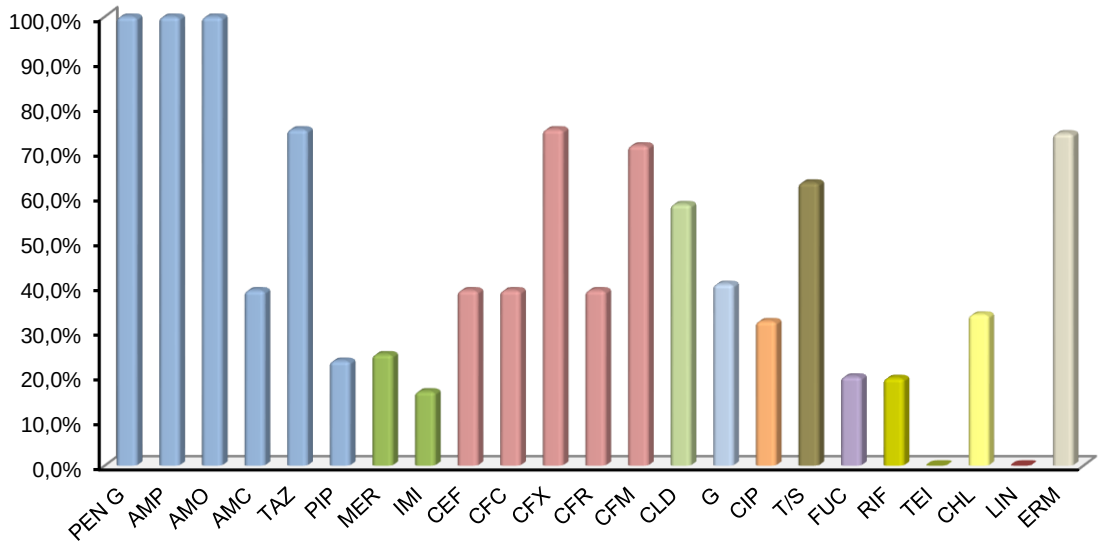

Fig. 2. Frequency of antibiotic resistance of *S. epidermidis*

PEN G-penicillin, AMP-ampicillin, AMO-amoxicillin, AMC-amoxicillin/clavulanic acid, TAZ-tazobactam, PIP-piperacillin/tazobactam, MER-meropenem, IMI-imipenem, CEF-cefalexin, CFC-cefaclor, CFX-ceftriaxone, CFR-cefuroxime, CFM-cefotaxime, CLD-clindamicin, G-gentamicin, CIP-ciprofloxacin, T/S -trimethoprim/sulfametoxazol, FUC-fusidic acid, RIF-rifampicin, TEI-teicoplanin, CHL-chloramphenicol, LIN-linezolid, ERM-erythromicin

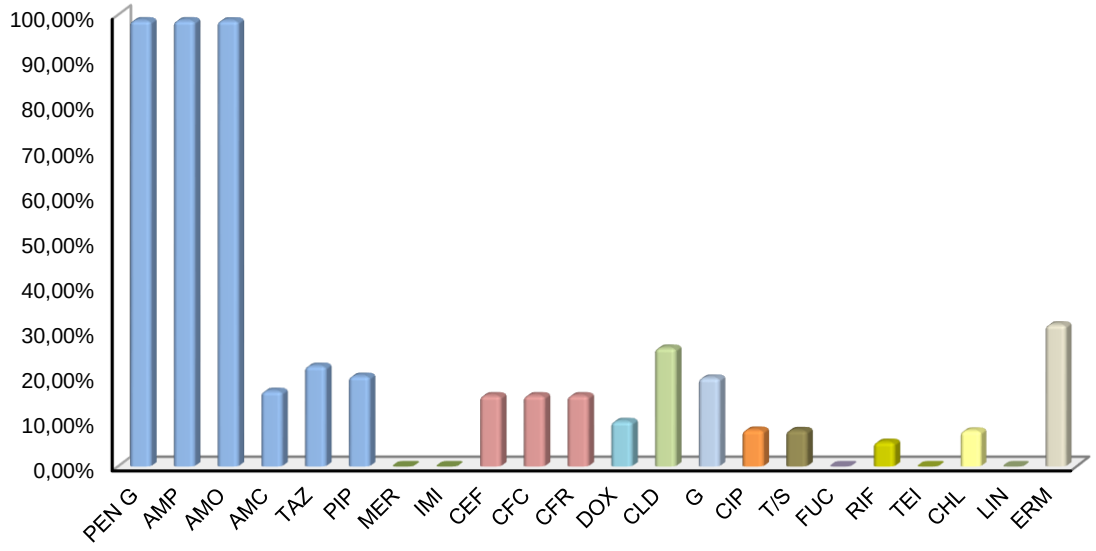

Fig. 3. Frequency of antibiotic resistance of *S. aureus*

PEN G-penicillin, AMP-ampicillin, AMO-amoxicillin, AMC-amoxicillin/clavulanic acid, TAZ-tazobactam, PIP-piperacillin/tazobactam, MER-meropenem, IMI-imipenem, LIN-linezolid, CEF-cefalexin, CFC-cefaclor, CFR-cefuroxime, DOX-doxycicline, CHL-chlomphenicol, ERM-erythromicin, CLD-clindamicin, G-gentamicin, CIP-ciprofloxacin, T/S-trimethoprim/sulfametoxazol, FUC-fusidic acid, RIF-rifampicin, TEI-teicoplanin

Pondei et al., 2013) in the same country, showed significant changes in these frequencies, where *P. aeruginosa* strains were the most dominant (33.3% and 32.6%, respectively). Considering studies performed in Serbia (Stanković-Nedeljković et al., 2012; Jovanović et al., 2014), which reported Gram negative bacteria *P. aeruginosa* and *E. coli* to be the most prevalent ones, there is also high disagreement with the obtained results in this study, where two Gram positive strains presented those with the highest isolation frequency.

The isolates of *S. epidermidis* were absolutely resistant (100.0%) to the penicillin antibiotics (penicillin G, ampicillin and amoxicillin), while a higher percentage of isolates showed sensitivity to amoxicillin/clavulanic acid and piperacillin. The carbapenems were significantly effective against the tested strains *of S. epidermidis*. The absolute

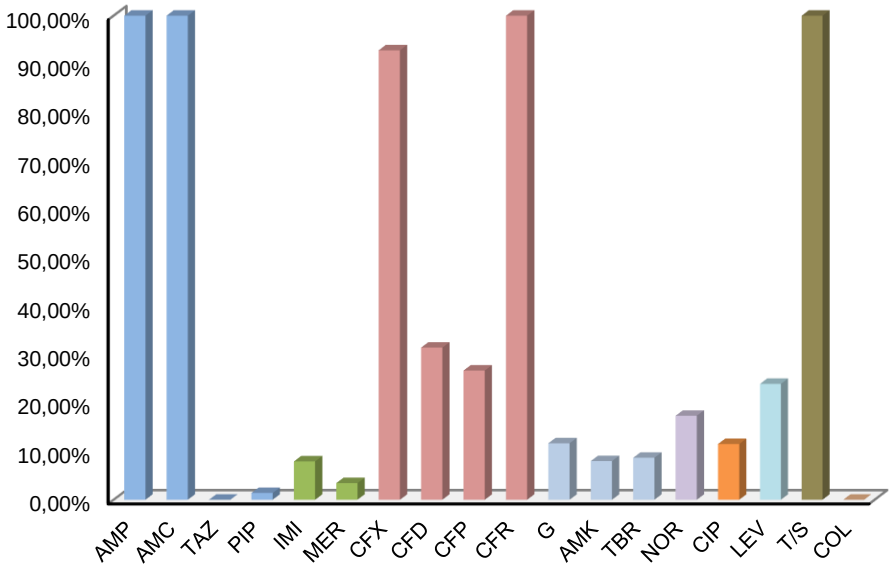

Fig. 4. Frequency of antibiotic resistance of *P. aeruginosa*

AMP-ampicillin, AMC-amoxicillin/clavulanic acid, TAZ-tazobactam, PIP-piperacillin/tazobactam, IMI-imipenem, MER-meropenem, CFX-ceftriaxone, CFD-ceftazidime, CFP-cefepime, CFR-cefuroxime, G-gentamicin, AMK-amikacin, TBR-tobramycin, NOR-norfloxacin, CIP-ciprofloxacin, LEV-levofloxacin, T/S-trimethoprim/sulfametoxazol, COL-colistin

sensitivity was found in the cases of glycopeptide antibiotics (teicoplanin) and linezolid (**Fig. 2**).

The results of research conducted in Mexico do not indicate similarity with the results of our study (Castro-Alarcon et al., 2011). In 2014, study conducted on *S. epidermidis* isolates obtained from intensive care unit patients showed the highest resistance to erythromycin (59.4%), tetracycline (57.8%) and trimethoprim-sulfamethoxazole

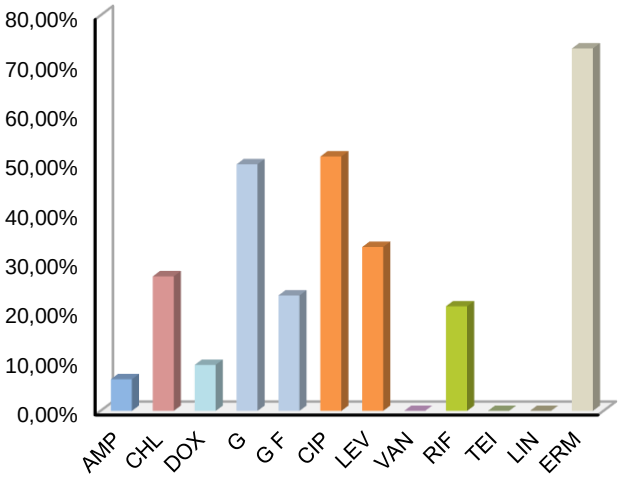

Fig. 5. Frequency of antibiotic resistance of *E. faecalis*

AMP-ampicillin, LIN-linezolid, DOX-doxycicline, CHL-chloramphenicol, ERM-erythromicin, G-gentamicin, G F-gentamicin forte, CIP-ciprofloxacin, LEV-levofloxacin, RIF-rifampicin, VAN-vancomycin, TEI-teicoplanin

(53.1%) (Najar-Peerayeh et al., 2014). In the present study, percentage of the strains resistant to erythromycin and trimethoprim-sulfamethoxazole were also very high (74.1% and 63.2%, respectively) and had the similar relation (trimethoprim-sulfamethoxazole demonstrated higher efficacy against isolates). Considering *S. aureus* isolates, resistance to ampicillin, penicillin G and amoxicillin, which all belong to penicillins, amounted 98.7% for all three antibiotics. Study of isolates obtained from the patients of the Instutute for Public Health in Ćuprija (Serbia) reported similar data, showing high resistance of these strains to penicillin group of antibiotics (Petrović-Jeremić et al., 2008). All tested strains of *S. aureus* were sensitive to carbapenems (imipenem and meropenem), linezolid, doxycycline, fusidic acid and teicoplanin (**Fig. 3**).

Investigation in Nigeria (Fadeyi et al., 2008) found the highest resistance of *S. aureus* isolates to fluoroquinolones, which was not the case in our study. Contrary to this, Sader et al. (2001) reported almost identical results with ours for the glycopeptide antibiotics (vancomycin and teicoplanin), which were the most active against all *S. aureus* isolates (100.0% susceptibility) in both studies.

Pseudomonas aeruginosa showed high rates of resistance to several classes of antibiotics (**Fig. 4**). In this study, we established the maximum resistance (100.0%) to ampicillin, amoxicillin/clavulanic acid, cefuroxime, and trimethoprim/sulfometaxazol, while slightly lower number of isolates (92.9%) showed resistance against ceftriaxone. Out of 78 *P.*

aeruginosa isolates, 7.9% were resistant to imipenem and even lower percentage to carbapenems (3.5%).

Carmeli et al. (1999) got similar results about the susceptibility of *P. aeruginosa* to different classes of antibiotics. Absolute sensitivity of the *P. aeruginosa* isolates has been observed toward tazobactam (penicillin group of antibiotics) and to polypeptide antibiotic colistin. Similar data were obtained by the research of nosocomial infection in Boston (Troillet et al., 1997).

The strains of *E. faecalis* were in the highest percentage resistant to erythromycin (73.3%), which belongs to the macrolide group of antibiotics (**Fig. 5**). The greatest sensitivity of the pathogen was determined to the glycopeptide antibiotics, vancomycin and teicoplanin, while all tested isolates of *E. faecalis* showed sensitivity to linezolid.

All tested isolates were sensitive to vancomycin which is highly important, bearing in mind the fact that occurrence of multi-resistant enterococci, in particular vancomycin - resistant enterococci (VRE) is continuously reported (Rice, 2001). In comparison to the survey conducted in Kuwait (Udo et al., 2002), there was a great similarity in the results.

Examination of individual resistance of all other isolated bacteria wasn't the part of the reported results of this research, because of their low isolation frequency from wound swabs and, therefore, low number for analysis performance. A small number of isolates wouldn't give a true picture of the resistance/susceptibility of bacteria to different drugs. These isolates were taken into account in determining the total resistance of Gram-positive and Gram-negative bacteria to various classes of antibiotics (**Fig. 6**).

Significant difference in the tetracycline efficiency was found when the resistance patterns of Gram-negative and Gram-positive bacterial strains were compared. Gram-negative bacilli were resistant to tetracycline in 60.8% of samples, while Gram-positive cocci showed lower resistance (15.1%). The carbapenems were the most active against most of the Gram-positive and Gram-negative bacteria (>80.0% susceptible). These results are similar with research in Uganda (Anguzu & Olila, 2007).

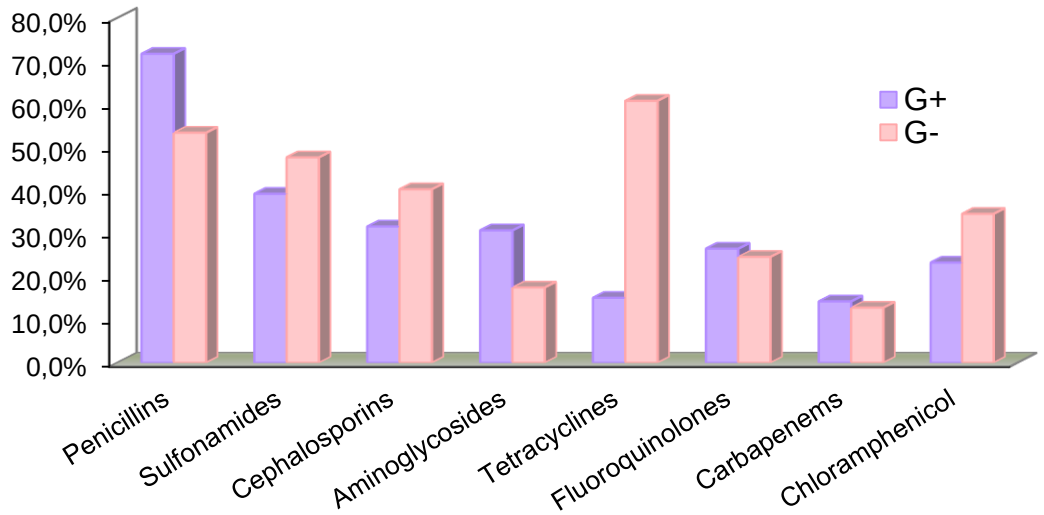

Fig. 6. Comparison of the resistance patterns of Gram-negative and Gram-positive bacterial wound isolates

Conclusion

A total of 613 bacterial isolates were isolated and identified from 526 samples of wound swabs. The most common causative agents of wound infection were the members of the genus *Staphylococcus*. *Staphylococcus epidermidis* was the predominant species (18.4%), followed by *S. aureus* (16.8%), *P. aeruginosa* (12.7%) and *E. faecalis* (10.4%). The Gram-positive cocci showed the highest resistance rates to penicillin, while Gram-negative bacteria were mostly resistant to tetracycline. Significant difference in the tetracycline efficiency was found when comparing the resistance patterns of Gram-negative and Gram-positive bacterial strains. According to the obtained results, antibiotic therapy of wound infections in Niš and surrounding area should always include previous isolation and antibiogram of the pathogen, but when it is not possible, the treatment of choice should include carbapenems or their combination with tetracyclines in order to cover both possible groups of causative organisms. At the same time, this approach leaves a battery of the last-resort antibiotics (e.g. linezolid,

teicoplanin) for each group of bacteria, which are found to be very efficient against obtained isolates.

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