Wound Infections with Multi-Drug Resistant Bacteria

H. Pîrvănescu¹, M. Bălășoiu², M.E. Ciurea¹, A.T. Bălășoiu³, R. Mănescu⁴

¹Department of Plastic Surgery, University of Medicine and Pharmacy, Craiova, Romania
²Department of Microbiology, University of Medicine and Pharmacy, Craiova, Romania
³PhD student, Department of Morphology, University of Medicine and Pharmacy, Craiova, Romania
⁴Department of Anatomy, University of Medicine and Pharmacy, Craiova, Romania

Abstract
Introduction: Wound infections remain a public health problem, despite the progress made on improving surgical techniques and antibiotic prophylaxis application. Misuse of antibiotics to prevent bacterial infections leads to increased bacterial resistance and their dissemination.

Material and Methods: The study refers to 470 samples taken from wound infections of which only multi-drug resistant strains were selected for study, using two special culture mediums (Metistaph-2 for methicillin-resistant staphylococci and ESBLs-Agar for extended-spectrum betalactamases secreting bacteria). Sensitivity of these strains was tested using the diffusion method.

Results: Of all studied samples, a rate of 27.6 bacterial strains showed multi-drug resistance. Among them stood primarily Staphylococcus aureus; both MRSA strains and ESBL Gram negative bacteria studied showed high resistance to aminoglycosides, quinolones, third generation cephalosporins and low to fourth generation cephalosporins. No vancomycin-resistant nor vancomycin-intermediate Staphylococcus aureus strains were isolated.

Conclusions: Knowing the antibiotic resistance is very useful in antibiotic “cycling” application, avoiding this way the emergence of increased resistant strains.

Key words: multi-drug resistance, methicillin-resistant Staphylococcus aureus, extended spectrum betalactamases
Introduction

Wound infections remain a public health problem despite the progress made on improving surgical techniques and applying antibiotic prophylaxis. Some studies suggest that antibiotic prophylaxis does not make any difference regarding wound infections incidence in patients with abdominal wall hernias (1). Risk factors depend on the host organism: age, disease severity, associated diseases, patient hygiene before surgery, extension of stay, postsurgical complications, immunosuppressive therapy or patient colonization with Staphylococcus aureus.

Bacteria have the ability to adapt to environmental conditions and resist to antibiotics, after overcoming and neutralizing immune barriers, followed by multiplication and invasion of the host organism.

The frequency of drug-resistant bacteria is increasing and includes original drug-sensitive bacteria. Drug-resistant bacteria tend to spread epidemically in hospitals and are involved in producing nosocomial infections (2,3). Misuse of antibiotics in order to prevent bacterial infections leads to an increased number of drug resistant bacterial strains. Unfortunately, a new series of antibiotics will not occur soon, whereas research and drug development require huge investments (that will be made only if future earnings are considered profitable for pharmaceutical companies).

WHO noted that unless terminated the misuse of antibiotics, they may not be effective when people need them. At this point, in the EU – Iceland and Norway – 25,000 people die every year because bacteria do not respond to antibiotics; most cases are in hospitals. Society might return to the moment when antibiotics were not discovered, when a simple infection could mean a death sentence, say experts in the field (4,5,6).

Antibiotics are always necessary in a bacterial infection, but abuse and misuse contribute to increased bacterial drug resistance. Studies show that many patients come with problems of antibiotic resistance because of inappropriate antibiotic use in the last six months. According to a study in progress at European level, Romania ranks third in antibiotic use, a position considered good. In 2009, Europeans consumed an average of 16 antibiotic doses per 1000 inhabitants, mostly for respiratory tract infection, and antimicrobial resistance (9).

A bacterial strain is considered as multi-drug resistant if it presents resistance to three antibiotics from different classes (quinolones, aminoglycosides, cephalosporins).

Based on the above assumptions we propose the study of multi-drug resistant (MDR) bacterial etiology in wound infections.

Material and Methods

The material was represented by 470 samples from different types of wounds (cuts, tears, burns and postoperative wounds) coming from the Plastic Surgery Clinic of the Emergency County Hospital of Craiova, in 2010 – 2011.

Wound infections have been evaluated depending on the risk of contamination with resistant or multi-drug resistant bacteria using Carmeli score (9).

Carmeli has recently introduced a new concept – infection associated to health care assistance, among the other two classic types of infection: nosocomial and community. Carmeli et.al. have published the first studies about bacterial antibiotic resistance (9).

Bacteriological diagnosis methods were complex. Thus, in the laboratory direct examination from product (wound), Giemsia and Gram stained was completed. Then samples were isolated on complex mediums: blood agar, both aerobic and anaerobic, CO2 atmosphere or Chapman.

Enterobacteriaeae identification was performed using conventional methods (API 20E bioMerieux standardized identification system) (10).

Chemotherapy sensitivity testing used A.W. Bauer standardized diffusion method, adopted by CLSI NCCLS USA. The medium used for antibiotic sensitivity testing for unassuming nutrient bacteria was Muller – Hinton.

For quality control we used the following reference strains: Staphylococcus aureus ATCC 25923 for testing Gram positive bacteria and negative control for betalactamase testing; Staphylococcus aureus 29213 as positive control for betalactamase testing; Escherichia coli (E. coli) ATCC 25922; Pseudomonas aeruginosa ATCC 27853.

Interpretation of inhibition zones produced on tested germ growth was sensitive, intermediate or resistant, in the presence of standard no. 0.5 National Committee for Clinical Laboratory Standards of McFarland barium sulphate scale (11,12,13).

Antibiotic selected sets were different depending on bacterial type and nutritional needs. For nutritionally demanding bacteria we have used a different range of antibiotics, in comparison to unassuming nutrient bacteria (National Guidelines for Application of Antimicrobial susceptibility testing according to CLSI/NCCSL Standard) (11,12,13,14,15).

The antibiotic selection was completed using also Carmeli score for bacterial infections: Carmeli 1 – community infection, Carmeli 2 – infection associated to health care assistance, Carmeli 3 – nosocomial infection (after 48 hours of admission to hospital).

Special tests

A. Screening for Methicillin-resistant Staphylococcus aureus (MRSA) was performed using Metistaph 2 medium. This medium is actually a Mueller-Hinton medium supplemented with ofloxacin on one half and with cefoxitin on the other half. On this medium only meticillin – resistant Staphylococcus aureus (MRSA) strains grow (10,11,14,15).

The confirmation test was performed using cefoxetine disc 30 μg for the 0.5 McFarland inoculum in diffusimetric antibiogram method. A diameter of inhibition less than 19 mm proves methicillin resistance (10,11,15).

B. Screening for Extended-spectrum betalactamases
(ESBL) testing was performed using ChromID ESBL AGAR medium. This medium is used for the isolation of ESBL Enterobacteriaceae. The confirmation was performed using the combined disk method, which consist in comparing the inhibition zone diameter on a disk of cephalosporin with and without clavulanic acid (10 μg of clavulanic acid added to the ceftazidime disk 30 μg). If the strain is ESBL positive, the inhibition zone for the disk with clavulanic acid increases with more than 5 mm, compared to the disk without inhibitor (11,12,13,14,15).

Results

From a total of 470 samples of wound secretions with positive bacteriological diagnosis, 130(27.6%) strains were multi-drug resistant (Carmeli 3), the other 340 (72.4%) were community strains (Carmeli 1).

MDR bacterial etiology was as follows:
1. Staphylococcus aureus – 54(41.5%) strains;
2. Enterococcus spp. – 5(4%) strains;
3. Enterobacteriaceae – 43(33%) strains (E. coli – 14, Klebsiella spp – 18, Proteus spp – 7, Enterobacter spp – 4);
4. Non-fermenting Gram negative bacteria – 28 (21.5%) strains (Pseudomonas spp – 25 strains, Acinetobacter spp – 3 strains);

1. Antibiotic resistance of MRSA

Resistance to aminoglycosides

MRSA strains showed resistance to aminoglycosides, MRSA associated with a rate of 48.8% (25 strains) KTG phenotype (kanamycin – tobramycin - gentamicin). Resistance to amikacin and netilmicin was lower, 16 (30.7%) strains were resistant to these antibiotics.

Resistance to quinolones

Resistance to fluoroquinolones was increased, namely:
- Ciprofloxacin – 21(38.8%) strains;
- Norfloxacin – 12(22.2%) strains;
- Ofloxacin – 7(12.9%) strains;
- Moxifloxacin – 6(11.1%) strains.

Resistance to rifampicin (reserve antibiotic for MRSA infections treatment) was low: only 2 (3.7%) strains were resistant. Mechanisms of resistance are mutations in the gene encoding RNA-polymerase.

Resistance to fourth generation cephalosporins (cefepime) was 16.6% (9 strains).

Resistance to trimethoprim-sulfamethoxazole. Only 6 (11%) MRSA strains were resistant to Biseptol, as a result of not using it for a long time.

Resistance to glycopeptides for MRSA is sporadic, few strains in the world presenting intermediate resistance or resistance to vancomycin (VISA, VRSA) and cross-resistance to teicoplanin. Our study showed no resistance (VRSA) and no intermediate resistance (VISA) to vancomycin and neither to teicoplanin (Fig. 1).

2. Antibiotic resistance of Enterococcus spp

Antibiotic resistance in enterococci is due to betalactamase production and penicillin-binding protein (PBP) modification. Resistance by betalactamase production is associated with resistance to gentamicin. Enterococcus strains have a natural resistance to lincosamides. Studies show that more and more Enterococcus spp strains show high resistance to aminoglycosides (KTG phenotype) (16,17).

Oxazolidinones are a class of antibiotics (linezolid was the first of its class) with good activity against enterococci, being active on Enterococcus faecalis and on Enterococcus faecium susceptible/resistant to glycopeptides. Identification of VRE (vancomycin-resistant Enterococcus spp.) at species level is necessary because it confirms if the isolated strain presents intrinsic (van C) or acquired (van A, van B) resistance. Knowing the type of resistance is essential because van A and van B genes are transferable and can spread from one organism to another. Instead van C genes are not transferable and are less involved in severe infections (11,18).

In our study the resistance to aminoglycosides was 80% (4 strains), to fourth generation cephalosporins 40% (2 strains) and to quinolones 20 - 40%. No Enterococcus spp. strains were resistant to rifampicin, vancomycin, teicoplanin or linezolid (Fig. 2).

3. Antibiotic resistance of fermenting Gram negative bacteria

Enterobacteriaceae represent a global problem which manifests through resistance to multiple drugs, the major cause being represented by extended-spectrum betalactamase (ESBL) production. The last decade has changed the ESBL structure. If by 2000 betalactamases were TEM (temoniera) and SHV type (sulphidril variable) and were associated with nosocomial infections, in 2000 CTX-M (cefotaximase M) became the main betalactamase, which confers resistance to third generation cephalosporins and which has spread in the community, particularly in E. coli (2,18,19).
ESBL spread has led to the increasing use of carbapenem-based antibiotics, leading to the emergence of other ESBL—the carbapenemase, a major therapeutic concern. A type of carbapenemase is the metal-beta-lactamase (MBL), which hydrolyses carbapenems and cephalosporins of all generations, but not the aztreonam. A new MBL was recently identified, namely New Delhi MBL-1 (NDM-1) in India, and it is present in Enterobacteriaceae infections acquired both in hospitals and in community. This metal-beta-lactamase can and will spread to other countries.

Actually, Gram negative bacteria use a combination of specific mechanisms (beta-lactamases, carbapenemases) and nonspecific mechanisms (efflux pumps, decreased porin expression) to express resistance.

### 3.1. Antibiotic resistance of ESBL Klebsiella pneumoniae

Of the 18 strains of Klebsiella pneumoniae, none showed resistance to imipenem, meropenem, ertapenem, amikacin and aztreonam, but showed resistance to gentamicin (8 strains - 44.4%), piperacillin/tazobactam (6 strains - 33.3%), trimethoprim-sulfamethoxazole (17 strains - 94.4%), ciprofloxacin (4 strains - 22.2%) and moxifloxacin (3 strains - 16.6%). Resistance to third generation cephalosporins (ceftazidim) was 100%, in comparison to resistance to fourth generation cephalosporins (cefepime), which was 27.7%. (Fig. 3)

### 3.2. Antibiotic resistance of ESBL E. coli

No E. coli strain showed resistance to imipenem, meropenem, ertapenem, aztreonam or amikacin. The rest of the antibiotic resistance was as follows: ciprofloxacin – 50%, moxifloxacin – 35.7%, cefepime – 28.5%, ceftazidim – 100%. We have observed an increased resistance to piperacillin/tazobactam (71.4%), despite the fact that IDSA (Infections Diseases Society of America) considers this antibiotic to be a reserve for Enterobacteriaceae ESBL+.

(Fig. 4)

### 4. Antibiotic resistance of non-fermenting Gram negative bacteria

#### 4.1. Antibiotic resistance of ESBL Pseudomonas aeruginosa strains

Pseudomonas aeruginosa, commonly found in wound
infections, especially in burns, is resistant to many antibiotics. Natural resistance to beta-lactamases is important and acquired resistance (enzymatic and non-enzymatic) is very common. The 25 strains of ESBL Pseudomonas aeruginosa met an increased sensitivity to colistin and aztreonam (only 12% resistant strains), otherwise Pseudomonas aeruginosa strains were resistant as follows: gentamicin – 68%, ciprofloxacin – 80%, moxifloxacin – 48%, amikacin – 60%, ceftazidim – 100%, cefepime – 60% (Fig. 7). Resistance to carbapenems was between 16% and 32%, in spite of the recent EARSS (European Antibiotic Resistance System Surveillance) studies, which indicate a resistance percentage of 50 to carbapenems for Romania, Greece and Bulgaria.

4.2. Antibiotic resistance of Acinetobacter baumanii strains

Acinetobacter baumanii, a commensal until recently, has become one of the most resistant germs that cause severe hospital (including wound) infections. The three ESBL+ Acinetobacter baumanii strains were resistant to third generation cephalosporins, aztreonam and third generation quinolones; to meropenem, imipenem, cefepime and moxifloxacin two strains were resistant. To colistin and ertapenem only one strain was resistant (Fig. 8).

Discussions

Bacteria involved in wound infections come from endogenous flora (especially Staphylococcus), favoured by invasive procedures or contaminating substrate used in the care of patients (21).

First in the etiology of wound infections was Staphylococcus aureus. Our study also reveals a shortage of Gram negative bacilli in the etiology of these infections, which is consistent with international studies.

Extensive use of antibiotics in hospitals, but also outside
them (feed, agriculture) contributed to the selection of resistant strains. MRSA increased resistance to all penicillins, cephalosporins, high percentage of resistance to quinolones and macrolides makes us use the linezolid in MRSA infections treatment. In time, however, long term use may lead to the emergence of linezolid – resistant MRSA strains (22, 23).

An encouraging aspect (for the moment) revealed by our study is the absence of vancomycin – resistant Staphylococcus aureus and vancomycin – resistant Enterococcus spp, although international studies have already indicated Staphylococcus aureus intermediary to vancomycin.

Gram negative bacteria producing extended spectrum beta-lactamases (ESBL) also showed resistance to third-generation cephalosporins, quinolones and even aztreonam. Carbapenems remain the only therapeutic option for ESBL + Gram negative bacteria.

The sensitivity of Pseudomonas aeruginosa and Acinetobacter baumanii to colistin made some authors believe that it is no longer appropriate to use carbapenems as first line therapy in infections with these germs. These authors propose as first line therapy in Pseudomonas aeruginosa and Acinetobacter baumanii infections colistin in association with other active antibiotics, in order to prevent the emergence of colistin – resistant strains (24).

Nimish Patel et al showed in a study published in the 48th Annual Reunion of IDSA (2010) that the use of ertapenem in Pseudomonas aeruginosa infections has reduced the Pseudomonas aeruginosa resistance to imipenem. The authors believe that this is possible because of the reducing of ciprofloxacin use and the decrease of the activity of the ciprofloxacin – induced efflux pump, which is linked to imipenem resistance. Authors suggest that the decrease of ciprofloxacin use could control the nosocomial infections with Pseudomonas aeruginosa resistant to cabapenems.

Some authors believe that in the empirical treatment of wound infections it is not recommended to start with anti-Pseudomonas drugs, because these drugs are not always necessary (25).

Knowing the antibiotic resistance is useful in applying the antibiotic “cycling” – periodic replacement of antibiotics with other antibiotics of the same family, but not exposed to resistance mechanisms.

Conclusions

MDR Enterobacteriaceae showed increased resistance to aminoglycosides, quinolones, trimethoprim-sulfamethoxazole, but not to imipenem, ertapenem, meropenem and aztreonam. Non-fermenting Gram negative bacteria showed the highest resistance to chemotherapy, some strains even to imipenem, meropenem, ertapenem, and aztreonam, but very few. This is why these four antibiotics, along with piperacillin-tazobactam remain reserve antibiotics for MDR Gram negative bacteria (fermenting and non-fermenting).

The strategy to reduce the risk of antibiotic resistance is represented by the reduction of bacterial exposure in clinical and community environment. This strategy can be accomplished by respecting measures to prevent infections and antibiotic administration with extreme caution.

The study must be filled by introducing large scale bacterial genotyping methods and can thus determine highly mutable subpopulations of pathogenic bacteria. These subpopulations have a higher rate of spontaneous mutation than most of the bacterial population due to defects in DNA replication and repair, becoming multi-drug resistant bacteria.

References

13. Performance Standards for Antimicrobial Susceptibility Testing; Sixteenth Informational Supplement. CLSI/NCCLS
document M 100-S16, 2006. CLSI 940 West Valley Road, Suite 1400, Wayne, PA 19087 / 1898 USA.


