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BACTERIA AND THEIR RESISTANCE TO ANTIBIOTICS IN THE INTENSIVE CARE UNIT

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ABSTRACT. In 2017, in the intensive care unit the bacteria incidence was bigger at the patients with invasive infections, intubated and catheterized: *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Escherichia coli* and MRSA. At most isolated bacteria species we have discovered a multi-resistance, remaining too few therapeutical options according to the antiobiogram. Nosocomial infections are continuing to represent a major problem for the patients safety and therefore introducing a programme for judicious prescriptions of antibiotics (antimicrobial stewardship) in a multidisciplinary hospital.
KEYWORDS: Bacteria, intensive care.

INTRODUCTION

The surveillance of antiobiotic resistance of bacterial isolates in the invasive infections from the pacients admitted into I.C.U. (intensive care unit) remains a major communitary health problem, both for establishing a targeted antibiotic treatment and for creating a fighting policy against gaining chemioresistance. The risk of infections associated with mechanical ventilation and central venous catheter, stays high despite the good practices of changing these medical devices (Balașa, 2012).

In Roumania, the antibiotic intake is excessive and increasing, and the exaggerated use of chimiotherapics with higher spectrum and those intestinal dismocrobism inductors (cephalosporins, quinolones, beta-lactamase inhibitors) in detriment of those with a small spectrum, neglected(trimethoprim + sulfamethoxazole, cyclines, phosphomycine, nitrofurantoin) (Popescu and colabs, 2014). The level of antibiotic resistance in Roumania, places us in top charts in Europe, for more pathogenic bacterial species (MRSA, *Pseudomonas aeruginosa*, *Acinetobacter baumanii*, *Klebsiella penumoniae*, *Escherichia coli*) (Popescu and colabs., 2014).

Gram negative bacilli and multiresistant Gram positive coci to antibiotics are frequently involved in nosocomial infections at patients with multiple comorbidities, intubated or catheterised, antibiotreated and imunosupressed. At european level, the combinated bacterial antibiotic resistance phenomenon records a on going ascesion for cephalosporines, fluoroquinolones, aminoglicosides and methicillin.

We assist at more and more frequent isolation of some bacterias from tracheobronsic secretion of the intrinsic resistant bacteria to colistin- *Providencia stuartii* (CLSI, 2017; EUCAST, 2017). The isolation of Gram negative MDR bacterias which are correlated with the development of some species with intrinsic resistance to colistin- *Providencia stuartii*, is caused by the increase of colistin consumption, carbapenems, tigecyline in mono or politheraphy (Szekely and colabs., 2016; Popescu, 2016).

AIM OF THE STUDY

To evaluate the aetiology and antibiotic resistance of isolated bacteria from tracheo-bronchial secretions and catheter peaks collected from invasive infections from patients admitted to the Intensive Care Unit (ICU) in the Arad County Emergency Clinical Hospital during the period January-December 2017.

MATERIAL AND METHODS

A total of 241 bacterial isolates from tracheo-bronchial secretions harvested with oro-tracheal intubation probes and 7 isolates from peaks were isolated, evaluated, microscopically evaluated, cultured on non-selective and selective culture media, identified and tested for antibiotics catheter. In interpreting the results we used classical methods and Vitek 2C automated system, according to Eucast (European Committee on Antimicrobian Susceptibility Testing), CLSI (Clinical and Laboratory Standards Institute-SUA) standards.

RESULTS

The pathology of invasive infections in patients included in the study includes:

- Surgical disease: neoplasm of perforated colon with secondary peritonitis (50%), perforated gastric ulcer with generalized peritonitis (15%), postoperative strangulated eventration with necrosis and perforation of the gut and secondary peritonitis (20%), appendicitis perforated with peritonitis at adults and children; inguinal hernia and strangulated umbilical with intestine necrosis and perforation (10%), and rarely perforated gangrenous gallbladder cholecystitis with biliary peritonitis (5%).
- Non-surgical diseases: chronic obstructive pulmonary disease (COPD), stage IV acute with community-acquired bronchopneumonia (BP), with evolution to nosocomial BP after noninvasive and

invasive mechanical ventilation (56%), ischemic and haemorrhagic stroke associated with community BP; (20%), vascular decompensated hepatic cirrhosis (CH) and superinfected ascites (10%), bronchial asthma, pulmonary fibrosis - superinfected (10%), grade II and III burns over 40 of body surface area, meningoencephalitis (2%).

Of the 248 investigated patients, 85% were mechanically ventilated because they had surgical and medical pathology, and 15% were not intubated.

In the study, we identified the 20th day for catheter change, correlating with changes in biological constants - hyperleucocytosis, procalcitonin (>20 ng/mL), local signs of infection, redness, and soreness; the catheter is also replaced with multiple therapeutic options, carbapenem + vancomycin + fluconazole, when after 2 weeks of medication the leukocytes grow in peripheral blood and procalcitonin. The oro-tracheal intubation probe was replaced on day 8-9 or whenever needed (in a patient with abundant tracheal exsudation, so on).

Of the 241 bacterial strains isolated from oro-tracheal probes, 175 are Gram negative bacilli and 66 Gram positive coci.

In the etiology of oro-tracheal intubation probe infections, 62 strains/ 25.72% of *Acinetobacter baumannii* and 25 strains of *Staphylococcus aureus* methicillin-resistant (MRSA). From the catheter tip are isolated frequently 2 strains of *Acinetobacter calcoaceticus* and 2 strains of *Staphylococcus hominis*.

In the etiology of Gram negative bacilli infections, 12 species were incriminated: *Acinetobacter (A.) baumannii*/ 62 strains, *Pseudomonas aeruginosa*/ 38 strains, *Klebsiella pneumoniae*/ 22 strains, *Proteus mirabilis*/ 13 strains, *Escherichia coli*/ 12 strains, *Acinetobacter calcoaceticus*/ 11 strains, *Providencia stuartii*/ 6 strains, *Klebsiella oxytoca*/ 3 and 2 strains of the species *Morganella morganii*, *Enterobacter cloacae*, *Stenotrophomonas maltophilia*, *Burkholderia cepacia*.

Gram-positive microbial strains isolated from tracheo-bronchial secretions were: MRSA / 25 strains, *Staphylococcus epidermidis*/ 17 strains, *Enterococcus sp.*/11 strains, *Staphylococcus haemolyticus*/ 6 strains, *Staphylococcus hominis*/ 5 strains and *Staphylococcus capitis* and *Staphylococcus cromogenes strains.*

In terms of microbial susceptibility, bacterial resistance (R) was predominant, 90%, to more than one representative of at least three different classes of antibiotics (multidrug-resistant bacteria, MDR). I isolated a pan-resistant bacillus strain from antimicrobial chemotherapies. At least 92% of the strains can be classified as nosocomial etiology. These infections occurred in patients with severe, major medical conditions requiring long-term hospitalization (on average - 15 days) with hospitalization in the ICU section with mechanical ventilation (85%). The treatment of these infections was costly and difficult, with an average duration of 10 days. 40% of patients died.

Of the 62 strains tested by *A. baumannii* all were carbapenem/ 100% resistant (imipenem, meropenem), cephalosporins of the third generation (ceftriaxone, ceftazidime)/ 100%, piperacillin + tazobactam/ 100% and trimethoprim + sulfamethoxazole/ 100 %. Different degrees of resistance to the other chemotherapies tested were found: 57 strains/ 91.93% aminoglycoside resistant isolates (gentamycin), fluoroquinolones - 52 resistant strains/ 83.87%, minocycline - 30 strains/ 48.38%. In colistin and tigecycline, resistance was the lowest, with 4 isolates strains/ 6.45%.

We identified patients with multibacterial infections, which associate with the initial etiology of *A. baumannii* other bacilli, such as: *Providencia stuartii*, *Burkholderia cepacia*, *Stenotrophomonas maltophilia*.

The resistance profile for the 38 strains of *Pseudomonas aeruginosa*, a germ that is often associated with medical care, looks like this: 18 strains resistant to carbapenems (imipenem, meropenem)/ 47.36%; to aminoglycosides (amikacin, gentamycin) – 17 strains/ 46.73%; 16 strains/ 42.10% for cephalosporins of III generation (ceftazidime, ceftriaxone), cephalosporins of IV generation (cefepime); 10 strains/ 26.31%/ fluoroquinolone-R (ciprofloxacin). In colistin I only isolated 2 resistant strains/ 5.26%, the pyocyaneus still remaining susceptible.

And in the case of some *Pseudomonas aeruginosa* infections, we noticed the association with *Providencia stuartii* (2 cases).

The 22 isolates of *Klebsiella pneumoniae* are resistant to antibiotics as it follows: to cephalosporins of the third generation (ceftriaxone, cefotaxime) - 11 strains/ 50%; aminoglycosides (amikacin, gentamycin) - 8 strains/ 36,36%; to fluoroquinolones (ciprofloxacin) – 8 strains/ 36,36%; betalactamins associated with beta-lactamase inhibitors (amoxicilin+clavulanic acid) - 8 strains/ 36,36%. We noticed a low significant degree of resistance in vitro to colistin - 5 strains/ 22,72%, tetracycline (tigecycline) - 2 strains/ 9,09% and carbapenem (imipenem, meropenem) - 2 strains/ 9,09%.

Phenotypic resistance to the third generation cephalosporins, fluoroquinolones and betalactamins associated with beta-lactamase inhibitors (amoxicilin+clavulanic acid), had 7 strains/ 53.84% of *Proteus mirabilis*. Pan-resistance (RDA) to antimicrobial is present in a strain of *Proteus mirabilis.*

Resistance of *Escherichia coli* bacillus to antimicrobial agents was: 7 strains/ 58,33% ceftriaxone; 5 strains/ 41,66% - ciprofloxacin; 3 strains/ 25% - amoxicilin + clavulanic acid; one strain/ 8,33% - carbapenems and aminoglycosides. No strains of tigecycline-resistant *E. coli* and piperacillin-tazobactam appeared.

The resistance profile of the 11 isolated strains of *Acinetobacter calcoaceticus* indicates elevated levels in most tested antimicrobials: 100% in the third generation cephalosporins and trimethoprim + sulfamethoxazole; 10 strains / 90,90% to IV and tigecycline cephalosporins, but they are susceptible to colistin; 9 fluoroquinolone strains/ 81,81% ; the same 8 strains/ 72,72% are resistant to aminoglycosides and carbapenems.

Particularly pleasing is the preservation of colistin susceptibility, as we have noted the association of colistin-resistant strains, in the case of a patient with

a plurimicrobial infection - *Acinetobacter calcoaceticus* + *Providencia stuartii* + *Proteus mirabilis* + *Enterococcus faecalis*.

We identified 9 patients with bacterial co-infections in the tracheo-bronchial secretions: a coinfection patient with 4 species, *Acinetobacter calcoaceticus* + *Providencia stuartii* + *Proteus mirabilis* + *Enterococcus faecalis*; coinfection with 3 species per patient, *Acinetobacter baumannii* + *Providencia stuartii* + *Stenotrophomonas maltophilia*. A total of 7 patients co-infected with 2 species, of which 4 patients were associated with *A. baumannii* + *Providencia stuartii*, 2 patients with *Pseudomonas aeruginosa* + *Providencia stuartii* and one board with *A. baumannii* + *Burkholderia cepacia*.

The presence of MRSA strains was found in all isolates/ 100% of the 25 strains of *Staphylococcus aureus* tested, similar to tetracycline and penicillin. Other resistance phenotypes present in *Staphylococcus aureus* isolates are: 13 strains/ 52% / erythromycin-R and clindamycin-R; 9 strains/ 36%/ tigecycline-R; one strain/4%/trimethoprim + sulfamethoxazole-R strain and fluoroquinolone-R. No MRSA resistance phenotypes were isolated from teicoplanin, linezolid, vancomycin.

All 6 strains /100% of *Staphylococcus haemolyticus* are resistant to penicillin, erythromycin, tetracycline, fluoroquinolones. All strains are non-susceptible to gentamicin, linezolid, vancomycin, tigecycline, teicoplanin. Only two strains of *Staphylococcus haemolyticus* methicillin-resistant (MRSCN) were obtained.

Staphylococcus (S.) hominis occupies the 3rd place as an opportunistic agent for *Staphylococcus aureus* and *Staphylococcus haemolyticus* infections with coagulase-negative staphylococci (SCN) and in our study occupies the same position if it suspects the actual presence of *Staphylococcus epidermidis*, a skin contaminant. All strains of S. hominis are MRSCN/ 100% (methicillin-resistant coagulase-negative staphylococci), fluorochinolone-resistant/ 100%, but also 100% to penicillin, clindamycin and erythromycin. Increased resistance levels were also found in phospho-mycin, 4 strains/ 80% and trimethoprim + sulfamethoxazole - 4/ 80%. Only one isolate of *S. hominis* is resistant to aminoglycosides, teicoplanin, tigecycline. The fact is that all strains are susceptible to linezolid and vancomycin because linezolid is considered a reserve antibiotic in MRSCN infections.

Other staphylococci and enterococci isolated in oro-tracheal probes have negligible resistance to the antimicrobial agents tested.

Of the total of 7 catheter infections, four were caused by Gram-positive *Staphylococcus hominis*/ 2 strains, MRSA/ 1 strain, *Enterococcus faecalis*/ 1 strain and three had as their etiology Gram negative bacilli - *Acinetobacter calcoaceticus*/ 2 strains, *Proteus mirabilis*/ 1 strain.

The 2 isolates of *Acinetobacter calcoaceticus* are pan-resistant, PDR (fluoroquinolones, carbapenems, aminoglycosides, tigecycline) and are only susceptible to colistin.

Proteus mirabilis isolate is resistant to carbapenems.

MRSA is also resistant to fluoroquinolones, clindamycin, erythromycin, aminoglycosides, penicillin.

Staphylococcus hominis 2 strains/ MRSCN also show resistance to other antimicrobials, such as erythromycin, clindamycin, aminoglycosides, fosfomycin, tetracycline. Sensitivity to linezolid, vancomycin, tigecycline is preserved.

DISCUSSIONS

The literature recommends changing the oro-tracheal intubation probe to 7 days or even tracheostoma.

In the catheterization situation, the catheter line changes in the following situations when there are objective factors: catheter infection suspected of fever, erythema, local purulent secretions, positive bacterial cultures, non-sterile manipulation, catheter thrombosis, not recommended for a certain amount of time, 10-14 days (Balasa, 2012).

Non-fermentative Gram negative bacillus *A. baumannii*, known as the etiologic agent of nosocomial infections, often met and with a high risk of damage, especially in patients with prolonged hospitalizations and invasive maneuvers, selected after long-term antibiotic treatments, records in our study multi-resistant to most antibiotics tested and a high degree of non-susceptibility (100%), resulting their ineffectiveness. These results are comparable to data reported in Romania in 2014 (Popescu, 2014). Antibiotics such as colistin and tigecycline are salvage for these patients.

Pseudomonas aeruginosa, a non-fermentative ubiquitous gram-negative bacillus, is provided with an external membrane that is difficult to cross and has intrinsic resistance to several classes of antibiotics (Szekely, 2016; Popescu, 2016). Antibiotics tested with the recommended antipseudomonadic action are few: beta-lactamases such as cephalosporins (ceftazidime, ceftriaxone, cefepime) and combinations with beta-lactamases inhibitors (piperacillin-tazobactam), fluoroquinolones (ciprofloxacin, levofloxacin), aminoglycosides (gentamicin, amikacin), carbapenems (imipenem, meropenem), colistin (Popescu, 2016).

In our study, we recorded a significant degree of resistance of *Pseudomonas aeruginosa*, approximately 50%, to carbapenems, aminoglycosides, cephalosporins III and IV, comparable to data reported in 2014 (Popescu, 2014).

The resistance of *Klebsiella pneumoniae* strains to carbapenems, has increased in recent years, but also its ability to secrete carbapenemases and to transfer genetic elements to other related *Enterobacteriaceae* is a major problem in the treatment of infections with this bacterium.

The results obtained are comparable to the reports of Romania and other studies and confirm important levels of resistance of *Klebsiella pneumoniae* to cephalosporin III, aminoglycosides, fluoroquinolones, betalactamase inhibitors and significantly to colistin (Popescu, 2014). Therapeutic variants remain tigecycline and carbapenems.

The data of our study confirms the evolutionary trends in recent years in Romania, as fluoroquinolones, commonly administered in *Escherichia coli* infections, are useful in about 50% of cases.

In penumonias the „Angelescu Guide" recommends, the following drug associations:

- In late nosocomial pneumonia or severe forms with *Pseudomonas aeruginosa*, *Acinetobacter*, *MRSA:* anti*pyocyaneus* beta lactamines/ colistin + aminoglycosides, anti*pyocyaneus*/ flouroquinolones +/- linezolid/ vancomycin then the adaptation of the therapeutical scheme according to the etiology and the antibiogram, as: colistin to resistant *Pseudomonas aeruginosa* to carbapenems, colistin + tigecycline to *Acinetobacter*, linezolid/ vancomycin to *MRSA* (Benea and colabs., 2012);
- In penumonia at neutropenic immunosuppressed pacients with staphylococcus infections, the Gram negative bacilli (inclusive piocianic): anti*pyocyaneus* beta lactamines + intravenous respiratory flouroquinolone + topic aminoglycoside anti*pyocyaneus* betalactamines + aminoglycosides + macrolide (Benea and colabs., 2012);
- In pneumonia at immunosuppressed pacients with MRSA infections, the Gram negative bacilli: cephalosporins of third generation/amoxi-inhibitors of beta lactamases + macrolide or intravenous respiratory flouroquinolone + vancomycin + rifampicin/ linezolid (MRSA) or intravenous respiratory flouroquinolone+ active betalacatamine on Gram negative bacilli + aminoglycoside (*Enterobacteriaceae*) (Benea and colabs., 2012).

We report the selection of infections / coinfections with non-fermenting Gram-negative bacilus type *Providencia stuartii*, *Morganella morganii*, *Stenotrophomonas maltophilia*, *Burkholderia cepacia* and possible etiological cause, correlation with the treatment with colistin and tigecycline of the primoinfection.These isolated bacteria are still a problem and by the non-susceptibility to the vast majority of the antibiotic classes used, including the third generation cephalosporins, but susceptible to IV generations of cephalosporins and intrinsic resistance, with few therapeutic options remaining (Szekely, 2014).

The microorganisms *Providencia stuartii*, *Morganella morganii*, *Stenotrophomonas maltophilia*, *Burkholderia cepacia* have intrinsic resistance to the often used antibiotics in infections with multiresistant bacilli (look at the table).

Table: The intrinsic resistance of some non-fermentative Gram negative bacillus, according to Eucast and CLSI

Bacterian species	Colistin	Tigecycline	Cephalosporins III	Meropenem/Imipenem
Providencia stuartii	R (Eucast/ CLSI)	R (Eucast/ CLSI)	___	___
Morganella morganii	R (Eucast/ CLSI)	R (Eucast/ CLSI)	___	___
Stenotrophomonas maltophilia	R (Eucast)	___	R (Eucast/ CLSI)	R (Eucast/ CLSI)
Burkholderia cepacia	R (Eucast/ CLSI)	___	R (Eucast/ CLSI)	___

R-resistant

The errors in using antibiotics are many (inappropiate associations or useless antibiotics: cocktail or „antibiotic bath", and so on) and microbial resistance represents in our days „the first problem" of antiinfectious treatment. Some studies proves the effectiveness of drug associations (meropenem+colistin) in running the test of synergy using the Etest strips impregnated with meropenem and colistin in increased concetrations, and the optimal MIC:MIC to values ≤ 0.5, condition to be applied in the microbiology laboratory (Pankey et al., 2013, www.biomerieux.com).

In our study we confirm the high degree of methicillin-resistance, penicillin - resistance (100%). The undesirable situation is the isolation of MRSA resistant to tigecycline, the most likely cause being prolonged treatment with this tetracycline. I have not isolated strains of MRSA resistant to vancomycin and linezolid, anti-MRSA antibiotics.

Linezolid, vancomycin, teicoplanin are used against *Staphylococcus* or rescue antistatic agents that are indicated for the treatment of MRSA or MRSCN multiresistant infections. The results of our study confirm the data in the literature. In the treatment of central venous catheter infections caused by MRSA, specialists recommend that initial therapy should be done with vancomycin or teicoplanin, and the dosage should be adapted to the sensitivity of isolates according to the antibiogram (Popescu and colabs., 2016).

At patients in the intensive care unit, most bacterial isolates are MDR.

Predominant are *Acinetobacter baumannii* and MRSA.

Antibiotics most commonly used in invasive infections are colistin, tigecycline, carbapenems and vancomycin, linezolid.

In Europe and implicitly in Romania, microbial resistance to antibiotics has become a major public health problem. Because antibiotic resistance is increasingly implicit in the selection of antibiotic-resistant bacteria, the safety of patients in hospitals is questioned by public opinion and medical forums.

In 2016, several actions were implemented in Romania that should have encouraged rational and weighted use of antibiotics in order to limit the phenomenon of resistance. However, the situation of antibiotic usage, places Romania on the 4th place at the level of the European Union (EU). Thus, for our country, the report of the European Center for Disease Prevention and Control (ECDC) regarding the resistance of isolated

bacteria from serious, invasive infections indicates the following
(https://www.ecdc.eu):
- *Klebsiella pneumoniae* is resistant / intermediate susceptible to carbapenems / 35.9%. It occupies the 3rd place in the EU.
- *Pseudomonas aeruginosa* resistant to carbapenems, down from 66% to 52%, but still ranked No. 1 in the EU.
- *Acinetobacter sp* - multi-resistant to carbapenems, aminoglycosides and quinolones, is increasing/ 82.9%, ranked second in the EU.
- *Streptococcus pneumoniae* resistant to erythromycin, occupies position 3 in the EU (39%.) Resistant to penicillin. 1st place in the EU (41.1%).
- *Staphylococcus aureus* - the highest percentage of EU strains of MRSA (methicillin resistant)/ 50.5%. 1st place in the EU.
- *Enterococcus faecium* resistant to vancomycin / 39%. 1st place in the EU.

If we compare our results with official ones, we see the same resistance pattern for *Acinetobacter*, *Pseudomonas aeruginosa* and *Klebsiella*, and the resistance of staphylococcus seems to can not be dethroned.

There is not exist a perfect overlaying of microorganisms *in vivo* with *in vitro* behaviour: S (susceptible)-clinical success and R (resistant)-clinical faillure. „For infections with medium or higher seriousness it is considered appropriate the aproximation: S - 90% clinical success and R - 60% clinical success (rule 90 - 60)" (Benea and colabs., 2012).

Extending the antibiotic resistance phenomenon increases the cost per patient in the healthcare system. ECDC estimates that resistant bacteria infections add up to EU health costs of around € 1.5 billion / year (https://www.ecdc.eu).

CONCLUSIONS

1. Anti-infective treatment with colistin in mono or polytherapy (meropenem + colistin), according or not to the antibiogram, increases in time the risk of choosing pathogens with intrinsic resistance to colistin and tigecycline.
2. The use of tigecycline in mono (tigecycline) or polytherapy (meropenem + tigecycline) as the only option for anti-infective treatment was significantly increased .
3. It is needed to establish a rational use policy for antibiotics in all medical specialties, knowing that morbidity and mortality rates are rising, and medical costs are steadily rising due to therapeutic failures.
4. Infections associated with medicated care continue to be a serious national problem and at the same time a major emergency for the Romanian population.
5. Possible significant success of anti-infective treatment could be achieved by: dosing antibiotics into body fluids and administering them on pharmacokinetic criteria; the determination of bacterial antibiotics in a real, correct, complete manner; evaluating the effectiveness of antibiotic associations (colistin + carbapenems).

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