

CLASSIFICATION OF GRAM-NEGATIVE BACILLI ACCORDING TO THEIR RESISTANCE TO ANTIMICROBIALS IN AN INFECTIOUS DISEASE CLINIC

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ABSTRACT

Objectives. Classification on multiple criteria of Gram-negative bacilli (GNBs) according to antibiotic resistance.

Material and method. Retrospective study (January 2017-December 2018) carried out in the Infectious Diseases Clinic from Craiova; GNBs were identified using the Vitek 2 automated system, which subsequently established their sensitivity to antimicrobials; GNBs were classified based on an arbitrary score from 1 (minimum) to 5 (maximum) based on the multiple antibiotic resistance index (MAR), the percentage of multidrug resistant strains (MDR) and the percentage of extended resistance strains (XDR). The final classification represents the sum of the points awarded for each category considered.

Results. The following GNBs were considered: *Escherichia coli* (n = 720), *Klebsiella pneumoniae* (n = 335), *Pseudomonas aeruginosa* (n = 139), *Proteus mirabilis* (n = 60) and *Acinetobacter baumannii* (n = 29). MAR values are: *Acinetobacter baumannii* (Ab) – 0.6, *Proteus mirabilis* (Pm) – 0.52, *Pseudomonas aeruginosa* (Pa) – 0.51, *Klebsiella pneumoniae* (Kp) - 0.37 and *Escherichia coli* (Ec) – 0.23. The percentage of MDR strains is: Pm – 76.67%, Kp – 68.86%, Pa - 58.71%, Ec – 51.94% and Ab – 51.72%; XDR strains were identified for Ab - 17.24% and Pa – 6.47%. The final classification of GNBs is as follows: Pa – 12p, Ab - 11 p, Pm – 7p, Kp – 6p, Ec – 3p.

Conclusions. Depending on the resistance profile on multiple criteria, the classification of the studied Gram-negative bacteria is as follows: Pa, Ab, Pm, Kp, Ec.

Keywords: Gram-negative bacilli, MAR, MDR, XDR

BACKGROUND

Gram-negative bacilli are an important category of microorganisms, responsible for human infections located in various sites such as the meningeal space, lungs, urinary tract, intra-abdominal space, bones, joints or wounds. Along with Gram-positive bacteria (particularly *Staphylococcus* spp.) they are the most important category of microorganisms responsible for sepsis / septicemia and are also involved in health-care associated infections [1-3].

Antimicrobial resistance is considered one of the greatest threats to humanity (alongside the climate changes), it is worldwide spreaded (with varying degrees) and it was recognized as a major health prob-

lem by the United Nations in a special meeting (September 2016) [4]. In 2017 the World Health Organisation (WHO) has published a list of pathogens for which new antibiotics are urgently needed due to their resistance to antibiotics [5]. On that list 5 out of 12 pathogens are Gram-negative bacilli and, more important, the first category (Critical) contains only this kind of germs (carbapenems resistant *Acinetobacter baumannii*, carbapenems resistant *Pseudomonas aeruginosa*, *Enterobacteriaceae* resistant to carbapenems or producing extensive spectrum betalactamases). More over, in 2015 plasmid-mediated polymyxin-resistance gene were discovered in China (polymyxins being considered “the last resort”

for the treatment of infections due to Gram-negative bacilli).

OBJECTIVE

The study aims to classify the Gram-negative bacilli (GNBs) isolated in an infectious disease clinic according to their resistance to antimicrobials, using multiple separation criteria.

MATERIAL AND METHODS

A retrospective study (January 2017 – December 2018) was conducted in the Infectious Disease Clinic from Craiova. The GNBs were identified using the Vitek 2 automated system, which subsequently established their susceptibility to antimicrobials. GNBs were classified based on an arbitrary score from 1 (minimum, given to the last ranked) to 5 (maximum, given to the first ranked), depending on the multiple index of antibiotic resistance (MAR), the percentage of multidrug resistant strains (MDR) and extended drug resistant stains (XDR). The final classification represents the sum of the points awarded for each category considered. The calculation of the MAR and the interpretation of results are based on the work of Krumpelman PH [7], while the criteria for defining MDR and, respectively, XDR for the GNBs are those proposed by Magiorakos et al [8]. Most strains were tested to 17 antibiotics and, in the presence of resistance to most of them, the testing was extended including another 9 antimicrobials. Commonly used drugs were: Amikacin, Ampicillin, Ampicillin-Sulbactam, Cefazolin, Cefepime, Cefoxitin, Ceftazidime, Ceftriaxone, Ciprofloxacin, Ertapenem, Gentamicin, Levofloxacin, Meropenem, Nitrofurantoin, Piperacillin, Tobramycin, Trimethoprim-Sulfamethoxazole. Extensive testing included: Aztreonam, Imipenem-Cilastatin, Minocycline, Pefloxacin, Piperacillin-Tazobactam, Polymixyn E, Ticarcillin, Ticarcillin-Clavulanic acid; Rifampicin was also introduced for the *Acinetobacter baumannii* strains.

RESULTS

Demographic data – The median age of patients was 60 years (limits: <1 year – 94 years). Most patients were adults (1197, 88.14%), female (701, 51.52%) and from urban areas (730, 53.75%).

We have identified 1358 strains of GNBs (see details in Table 1). For the purpose of the study we have

selected only the strains of *Escherichia coli* (Ec), *Klebsiella pneumoniae* (Kp), *Pseudomonas aeruginosa* (Pa), *Proteus mirabilis* (Pm), and *Acinetobacter baumannii* (Ab), which represent 1283 isolates (94.47% of the total).

TABLE 1. GNBs species and their count

GNBs	Number of isolates
<i>Escherichia coli</i>	720
<i>Klebsiella pneumoniae</i>	335
<i>Pseudomonas aeruginosa</i>	139
<i>Proteus mirabilis</i>	60
<i>Acinetobacter baumannii</i>	29
<i>Serratia marcescens</i>	11
<i>Klebsiella oxytoca</i>	9
<i>Enterobacter cloacae complex</i>	7
<i>Citrobacter freundii</i>	8
<i>Raoultella planticola</i>	5
<i>Raoultella ornithinolytica</i>	4
<i>Salmonella enterica</i>	4
<i>Sphingomonas paucimobilis</i>	4
<i>Morganella morganii</i>	4
<i>Burkholderia cepacia</i>	3
<i>Enterobacter aerogenes</i>	3
<i>Bordetella bronchiseptica</i>	2
<i>Stenotrophomonas maltophilia</i>	2
<i>Pantoea dispersa</i>	2
<i>Pseudomonas putida</i>	2
<i>Shigella sonnei</i>	1
<i>Leclercia adecarboxylata</i>	1
<i>Serratia plymuthica</i>	1
<i>Cronobacter sakazakii</i>	1
<i>Pantoea agglomerans</i>	1

The pathological products from which the GNBs were isolated are presented in Table 2.

TABLE 2. Pathological products and their count

Pathological product	Number of samples
Uroculture	619
Sputum	443
Coproculture	149
Blood culture	73
Wound secretion	36
Bronchial aspirate	14
Vaginal discharge	9
Ear discharge	7
Biliculture	2
Pericardial fluid	1
Cannula discharge	1
Probe discharge	1
Cerebrospinal fluid	1
Pleural fluid	1
Intraabdominal fluid	1

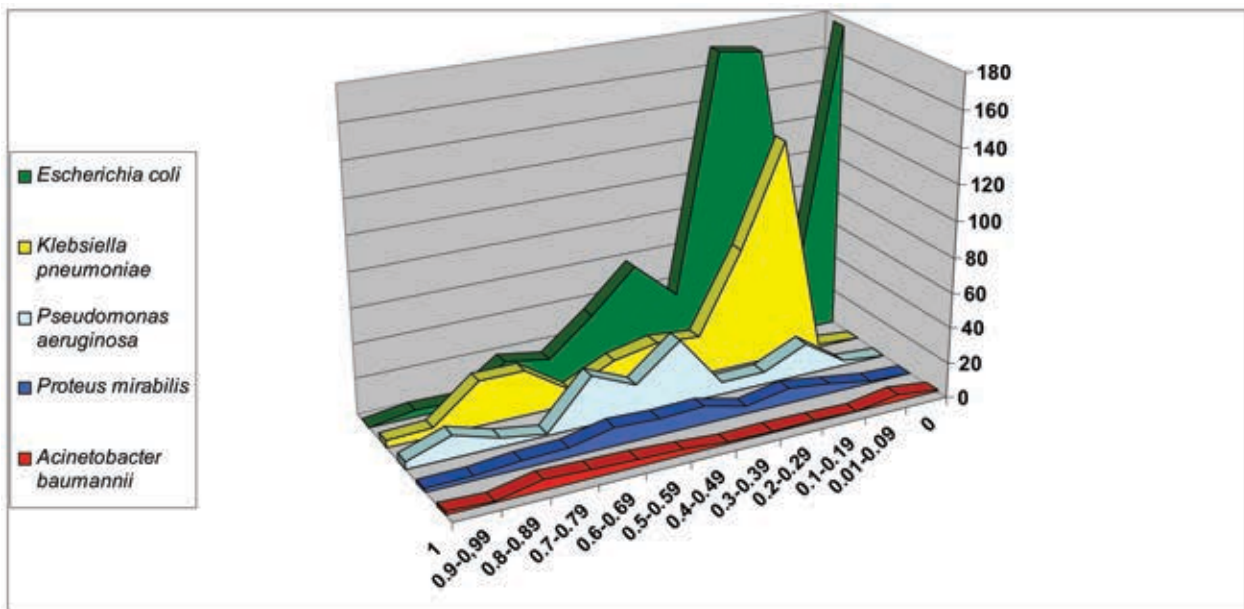


FIGURE 1. MAR index for the studied GNBs

For the first classification criteria (MAR index) the following data were obtained (mean±standard deviation): Ec – 0.23 ± 0.2 , Kp – 0.37 ± 0.25 , Pa – 0.51 ± 0.24 , Pm – 0.52 ± 0.24 , Ab – 0.6 ± 0.31 . For all the strains tested, the MAR index had an average value of 0.32 ± 0.25 . Figure 1 shows graphically the MAR index for each studied GNB strain.

For the 2nd and 3rd classification criteria (the percentage of MDR and XDR strains), the data are presented in Table 3.

TABLE 3. Percentage of MDR and XDR strains for the studied GNBs

GNBs	Ec	Kp	Pa	Pm	Ab
MDR (%)	51.94	68.86	76.67	58.71	51.72
XDR (%)	0	0	0	6.47	17.24

A summative graphical situation of the antimicrobial resistance problems identified for the studied strains is presented in figure 2.

The classification of the studied GNBs (according to their degree of antimicrobial resistance) is presented in Table 4.

DISCUSSION

The issue of resistance to antimicrobials is of extremely importance to any physician. Specialists know a number of "problematic bacteria" from this point of view: some strains of *Staphylococcus aureus*, *Enterococcus spp.*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella spp.* etc. Most of the antibiotic-resistant germs (at least according to

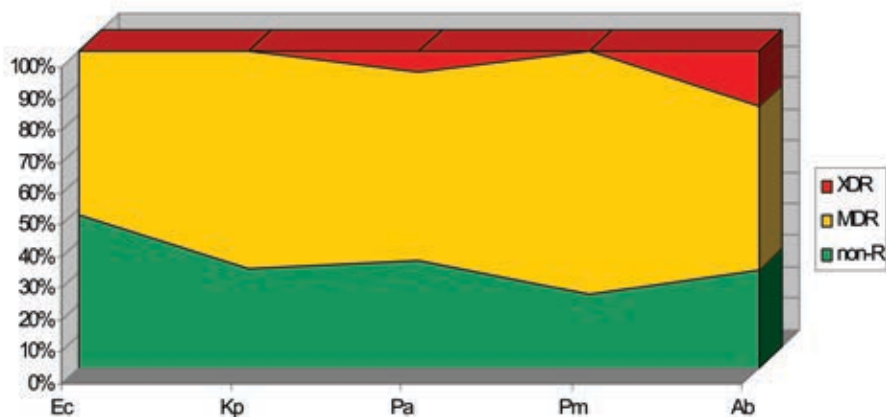


FIGURE 2. A composite graphic of the antimicrobial resistance problems for the studied GNBs

TABLE 4. Final ranking of the studied GNBs (NA = not awarded)

Criteria	Ec		Kp		Pa		Pm		Ab	
	Rank	Awarded points	Rank	Awarded points	Rank	Awarded points	Rank	Awarded points	Rank	Awarded points
MAR	5	1	4	2	3	3	2	4	1	5
%MDR	4	2	2	4	1	5	3	3	5	1
%XDR	NA	0	NA	0	2	4	NA	0	1	5
Total points		3		6		12		7		11

the WHO list) are Gram-negative bacteria. The author did not find data in the medical literature to classify these bacteria, to conclude "how much problematic" they are (at least in a certain geographical region). For the purpose of this paper an arbitrary scoring system has been used, but which, applied to each species, should not affect the final classification. One point of discussion could be the relevance of the classification related to the different number of strains isolated for each species, but the percentage expression for each classification criterion considered should be an objective point of analysis. It can also be argued whether the score should be identical

for each classification criterion; at least for the data presented, it was considered that each of them has an equal share in the final classification. This study is practically an exercise and can be useful, however perfectible in the future.

CONCLUSION

The final classification of Gram-negative bacteria analyzed in terms of antimicrobial resistance problems is as follows: 1. *Pseudomonas aeruginosa*; 2. *Acinetobacter baumannii*; 3. *Proteus mirabilis*; 4. *Klebsiella pneumoniae*; 5. *Escherichia coli*.

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