ORIGINAL PAPER

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

An overview of resistance profiles ESKAPE pathogens from 2010–2015 in a tertiary respiratory center in Romania

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Abstract

Lower respiratory tract infections (LRTIs) is an umbrella term that covers a wide spectrum of diseases, comprising mild and severe, acute and chronic conditions. A wide spectrum of pathogens can be implicated, from viruses to pyogenic and atypical bacteria. A special place should be reserved for slow growing bacteria (*Mycobacteria* spp., *Nocardia* spp.) and parasites (*i.e.*, hydatic cysts caused by *Echinococcus granulosus*). *Objective*: The objective of this study is to observe, analyze and establish the drug susceptibility patterns for *Enterococcus* spp., *Staphylococcus aureus*, *Klebsiella* spp., *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp. (the ESKAPE pathogens) in the "Marius Nasta" Institute for Pulmonary Medicine (MNIPM), Bucharest, Romania. *Materials and Methods*: A retrospective healthcare record based study was undertaken to establish the drug susceptibility patterns. We assessed all antibiograms of the ESKAPE pathogens isolated from respiratory samples from adult inpatients hospitalized between 2010–2015 at the MNIPM. *Results*: We analyzed 2859 isolates (61% of the 4683 ESKAPE isolates). *P. aeruginosa* was the most frequent pathogen, while *Enterococcus* spp. and *Enterobacter* spp. were practically non-present. The antibiotic profile of *P. aeruginosa* isolates presented more resistance in the Intensive Care Unit (ICU)/Surgery wards, probably resulting from antibiotic pressure. The other non-fermenter, *A. baumannii*, while less frequent (and the only pathogen more frequent in the surgery department) had an even more resistant profile, to almost all antibiotics, with the exception of Colistin. Methicillin-resistant *S. aureus* (MRSA) accounted for about 60% of all isolates, more in the ICU/Surgery ward. *K. pneumoniae* presents a less resistance and shows more stability when analyzing the antibiogram pattern in the Medical wards. *Discussion*: For methodological or procedural reasons, *Enterococcus* spp. and *Enterobacter* spp. were underrepresented in the study. Interventional programs comprising antibiotic stewardship and active surveillance need to be implemented to alleviate the antibiotic profile. Further research needs to focus on more detailed characterization of the molecular mechanisms leading to the high resistance detailed herein. *Conclusions*: This study adds to the body of literature reporting the antibiotic resistance landscape in Romania, for these highly resistant pathogens.

Keywords: lower respiratory tract infections, pneumonia, ESKAPE pathogens, antibiotic resistance.

Introduction

Lower respiratory tract infections (LRTIs) are a heterogeneous group of clinical entities that encompass extremely different pathologies. A wide variety of pathogens are implicated, from viruses to bacteria (pyogenic and atypical) and parasites. The ends of the spectrum, comprising mild and severe, acute and chronic conditions, can represent community and healthcare-associated/hospital-acquired infections.

Depending on predisposing factors and severity, community-acquired pneumonias (CAPs) can be diagnosed and treated in the primary care/ambulatory or inpatient setting. These rarely implicate resistant microorganisms, in the absence of predisposing factors (underlying pathology, corticoid treatment, etc.) [1]. Chronic obstructive pulmonary disease (COPD) is a chronic condition that often requires medical interventions (antibiotics, corticoids, hospital admission). Independently, diseases severity and the presence of underlying bronchial pathology (bronchiectasis) predisposes to opportunistic, sometimes drug resistant infections [2]. These patients may harbor resistant organisms and even when coming from a community setting. On the other hand, hospital- or healthcare associated pneumonia may be caused by resistant germs. Early-onset pneumonia is associated with fewer resistance, because of lack of colonization with hospital microbiota [3]. From five days onward (late-onset pneumonia), risks of antibiotic-resistant bacteria grows. This also includes ventilator-associated pneumonia [4].

In the US, the *Infectious Disease Society of America* (IDSA) and the *American College of Chest Physicians* (ACCP) have provided countrywide diagnosis and treatment guidelines, for community as well as healthcare-associated and nosocomial pneumonia [5].

Worldwide, *Enterococcus* spp., *Staphylococcus aureus*, *Klebsiella* spp., *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp. (ESKAPE) pathogens have

been associated with more severe infections, increasing morbidity and mortality as well as the costs associated with their treatment (tied to more hospital days, increased personnel and medication costs) [6–9]. The emerging multidrug resistance of these pathogens raises concern about the future of treatment options as well as clinical outcomes [10–13].

In Europe, national guidelines have more limited girth, as is the case of antibiotic prescription guidelines [14, 15]. In Romania, the epidemiology of LRTIs is not well characterized, due to few studies that have evaluated the subject [16]. Studies show increased antibiotic resistance in diverse healthcare settings in Romania [17–19].

The latest guidelines published by the *European Respiratory Society* (ERS) point out the importance of hospital-wide microbial ecology [20]. Knowing the hospital-specific resistance profile can lead to better empiric antibiotic choice and potentially improve major patient-centric endpoints such as morbidity and mortality.

The objective of this study is to characterize the implication of ESKAPE pathogens and their resistance in a tertiary respiratory care center in Romania. To our knowledge, this is the first study to characterize the hospital-wide microbiology ecosystem to span such a long duration of time.

Materials and Methods

A retrospective healthcare record based study was undertaken to establish the drug susceptibility patterns for the pathogens most likely to cause severe infections and develop antimicrobial resistance. We assessed all antibiograms of the ESKAPE pathogens obtained between 2010 and 2015 from the "Marius Nasta" Institute for Pulmonary Medicine (MNIPM), Bucharest, Romania.

All drug susceptibility testing was done by Kirby–Bauer method on Müller–Hinton agar medium. Regardless of the antibiotic breakpoints used for the interpretation in clinical practice, the inhibition zone analysis was done according to the 2016 *European Committee on Antimicrobial Susceptibility Testing* (EUCAST) and *Clinical & Laboratory Standards Institute* (CLSI) breakpoints.

Adult (>18-year-old) inpatient samples were selected for the analysis. The antibiograms were independently reviewed by two authors (NOP and AAM). Only respiratory samples were considered as per the laboratory sample entry database (this included sputum, induced sputum, bronchial aspirate, bronchial lavage, bronchial brush samples). Multiple entries pertaining to the same individual were removed. When multiple different isolates were recorded for the same patient, either simultaneously or sequentially, authors assessed patients' medical health record to establish the clinical consequence of the isolates. If antibiotic therapy was started or modified to cover the new/multiple pathogen(s), then the isolate(s) were considered clinically significant. If not, the pathogen was considered non-clinically significant.

Processing was done using R (version 3.2.1) and R Studio (version 1.0.136). Categorical variables were interpreted using χ^2 (*chi*)-square and Fisher's exact tests.

Results

Between 2010 and 2015, the MNIPM Microbiology Laboratory recorded a total of 4683 isolates that belong to the ESKAPE category. After applying the selection criteria (isolates from adult inpatients from respiratory samples), 2859 (61%) isolates were analyzed (Figure 1).

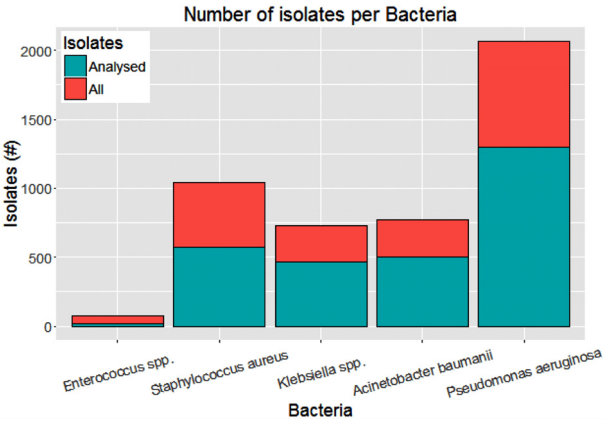

Figure 1 – ***Number of isolates that met inclusion criteria. A schematic representation of the strains analyzed versus all strains received by the MNIP microbiology laboratory. MNIP: "Marius Nasta" Institute for Pulmonary Medicine.***

Due to the very few isolates of *Enterobacter* spp. (n=2), we excluded them from final analysis, as they seem to play an infinitesimal role in our cohort of analyzed antibiograms.

Samples could not be consistently tested for all antibiotics recommended, and as such, we report the absolute numbers tested for each antibiotic, as well as the percentage of strains considered susceptible, intermediate or resistant.

The number of isolates per year of the different bacterial species and the number analyzed are outlined in Table 1.

Table 1 – ***The number of ESKAP strains (as Enterobacter spp. were too few to even index) isolated per year, the absolute number, as well as the percentage of strains analyzed***

Year	*Enterococcus* spp.		*Staphylococcus aureus*		*Klebsiella* spp.		*Acinetobacter baumannii*		*Pseudomonas aeruginosa*	
	All	Analyzed (%)	All	Analyzed (%)	All	Analyzed (%)	All	Analyzed (%)	All	Analyzed (%)
2010	15	4 (26%)	198	102 (51%)	129	78 (60%)	112	87 (77%)	403	244 (60%)
2011	12	2 (16%)	128	71 (55%)	99	63 (63%)	131	86 (65%)	275	173 (62%)
2012	5	1 (20%)	130	67 (51%)	140	72 (51%)	157	90 (57%)	295	202 (68%)
2013	20	4 (20%)	181	105 (58%)	113	78 (69%)	148	87 (58%)	311	212 (68%)
2014	15	7 (46%)	225	126 (56%)	161	110 (68%)	111	69 (62%)	410	239 (60%)
2015	9	3 (33%)	178	102 (57%)	85	65 (76%)	116	84 (72%)	371	226 (61%)

One of the first insights was that *Enterococcus* spp. and *Enterobacter* spp. were only rarely isolated from respiratory samples.

Enterococcus spp. was isolated in small numbers – median of 3 (min. 1–max. 7) – every year. The vast majority of isolates were encountered from urinary tract infections (UTIs) and so were not included in the analysis.

Staphylococcus aureus accounted for 1040 isolates between 2010 and 2015. After applying the selection criteria, 573 isolates belonging to adult inpatients, were included. A median of 102 (min. 67–max. 126) were analyzed each year.

A total of 727 isolates of *Klebsiella* spp. were recorded, out of which 466 met the eligibility criteria and were analyzed. A median of 75 (min. 63–max. 110) isolates were analyzed for every year of study.

Acinetobacter baumannii presented most often as an Intensive Care Unit (ICU) pathogen. The total number of isolates encompassed 775, out of which 503 represented valid samples for the study. A median of 87 (min. 69–max. 90) isolates were analyzed for every year of study.

Pseudomonas aeruginosa was consistently the most isolated of the ESKAPE pathogens, with an overall isolation of 2065 strains, 341 strains per year (min. 275–max. 410), out of which 1296 met the inclusion criteria, resulting in a median 219 strain isolations per year (min. 173–max. 244).

An important remark is that we identified only two *Enterobacter* spp. isolates in this time frame, which were excluded from the analysis. Number of yearly isolates was rather consistent throughout the years (Figure 2).

Isolates from the ICU and Surgery wards were aggregated due to the relative paucity of samples from the Surgery ward and the continuum of care within our Institution (Figure 3).

Enterococcus spp.

The number of isolates that seemed to cause infections is relatively low (n=21). This may be due to factors related to standard practice (the relatively few blood cultures taken) and methodology (difficulty in identifying enterococci among routine sputum isolates oftentimes contaminated with *viridans* streptococci).

In any case, from the relatively few isolates at our disposal, it is hard to draw relevant conclusions. We note that one isolated was Kirby–Bauer intermediate for Linezolid, and this should be confirmed by determining the minimum inhibitory concentration (MIC). The rest of the resistance profile is presented for reference (Table 2).

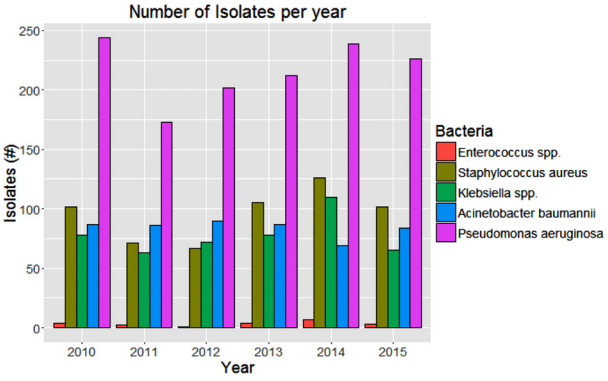

Figure 2 – ***Isolates analyzed by year. Histogram showing the number analyzed of each bacteria isolated each year.***

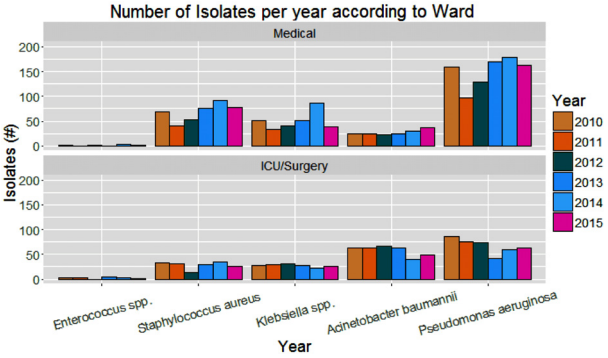

Figure 3 – ***Yearly isolates organized by ward. Histogram breakdown of the number of bacteria isolated from the Medical and ICU/Surgery wards, respectively, for each year of the study. ICU: Intensive Care Unit.***

Table 2 – ***The antibiograms for the Enterococcus spp. isolates***

***Enterococcus* spp.**			
CLSI	***R# (R%)***	***I# (I%)***	***S# (S%)***
AMP	8 (38.1%)	0 (0%)	13 (61.9%)
C	1 (5.26%)	1 (5.26%)	17 (89.47%)
CIP	10 (52.63%)	7 (36.84%)	2 (10.53%)
NOR	1 (100%)	0 (0%)	0 (0%)
DOX	4 (40%)	1 (10%)	5 (50%)
GN	4 (66.67%)	0 (0%)	2 (33.33%)
LZD	0 (0%)	1 (4.76%)	20 (95.24%)
VAN	0 (0%)	0 (0%)	18 (100%)
TEC	0 (0%)	0 (0%)	21 (100%)
E	9 (47.37%)	5 (26.32%)	5 (26.32%)

CLSI: Clinical & Laboratory Standards Institute; R: Resistant isolates; I: Intermediate isolates; S: Susceptible isolates; AMP: Ampicillin; C: Chloramphenicol; CIP: Ciprofloxacin; NOR: Norfloxacin; DOX: Doxycycline; GN: Gentamicin; LZD: Linezolid; VAN: Vancomycin; TEC: Teicoplanin; E: Erythromycin.

Staphylococcus aureus

ICU/Surgery samples accounted for roughly one-third (29%, n=167) of total isolates which fit the prespecified analysis criteria (n=573).

Both the EUCAST and CLSI guidelines have dropped Oxacillin (OXA) testing for the differentiation of Methicillin-resistant *S. aureus* (MRSA), opting instead for Cefoxitin (FOX) [21]. The majority of samples (68.74%, n=394)

were tested with Cefoxitin (FOX) for determination of MRSA status. Overall, Methicillin-resistant isolates were most often identified (58.12%, *n*=229) (Figure 4). There was a significant difference ($p<<0.001$) between the rates of MRSA samples recorded in the ICU/Surgery ward (85%, *n*=92) and in the Medical ward (47.9%, *n*=137) (Figure 5).

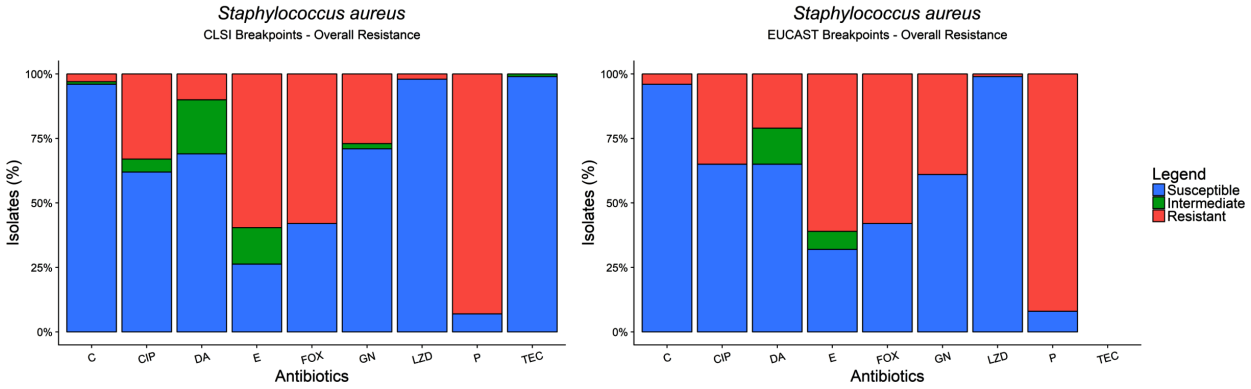

Figure 4 – ***Barplot showing the overall resistance of Staphylococcus aureus, when interpreting isolates as per the CLSI and EUCAST breakpoints respectively. CLSI: Clinical & Laboratory Standards Institute; EUCAST: European Committee on Antimicrobial Susceptibility Testing; C: Chloramphenicol; CIP: Ciprofloxacin; DA: Clindamycin; E: Erythromycin; FOX: Cefoxitin; GN: Gentamicin; LZD: Linezolid; P: Penicillin; TEC: Teicoplanin.***

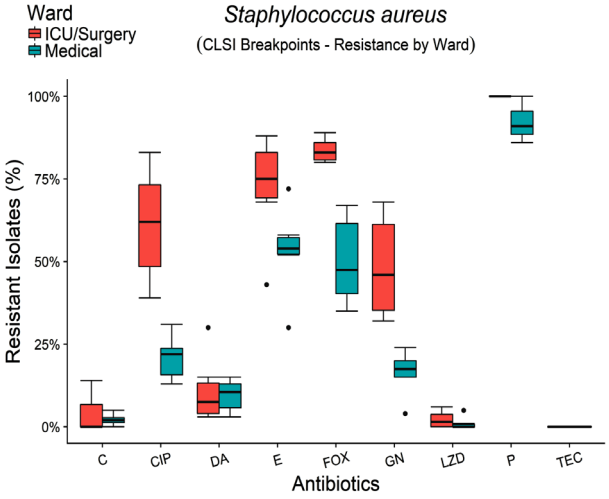

Figure 5 – ***Boxplots showing the variability of recorded resistance between isolates of Staphylococcus aureus received from the ICU/Surgery and the Medical ward. ICU: Intensive Care Unit; CLSI: Clinical & Laboratory Standards Institute; C: Chloramphenicol; CIP: Ciprofloxacin; DA: Clindamycin; E: Erythromycin; FOX: Cefoxitin; GN: Gentamicin; LZD: Linezolid; P: Penicillin; TEC: Teicoplanin.***

Overall resistance rates with difference between CLSI and EUCAST are presented in Table 3. Tables 4 and 5 present a breakdown of the resistance rates by wards, showing the number of isolates encountered, the number tested for each antibiotic and resistance rates expressed as percentages.

Penicillin can be used to treat sensitive Methicillin-susceptible *S. aureus* (MSSA) infections. The sensitive isolates are rare (<10%), but it could provide an affordable alternative in such infections.

Ciprofloxacin resistance was moderately high in the isolates tested – independent of the breakpoints applied – with >30% of the isolates showing resistance. Only small differences are present when comparing CLSI and EUCAST breakpoints, due to the presence of Ciprofloxacin intermediate strains, which accounted for about 5% (*n*=26).

Rifampicin susceptibility was not formally tested. Due to the high incidence of tuberculosis in Romania, Rifampicin is not a first line antibiotic treatment for such infections.

One notable difference due to breakpoint choice is found in the overall classification of Gentamicin resistance (39% *vs.* 27.2% – *n*=190 *vs.* 133, $p<0.01$) isolates resistant according to EUCAST and CLSI breakpoints respectively.

Table 3 – ***Breakpoint interpretations for Staphylococcus aureus for each of the antibiotics tested. CLSI and EUCAST interpretations side by side for comparison***

	Staphylococcus aureus					
Ab	**CLSI Overview**			**EUCAST Overview**		
	R# (R%)	***I# (I%)***	***S# (S%)***	***R# (R%)***	***I# (I%)***	***S# (S%)***
FOX	229 (58.12%)	0 (0%)	165 (41.88%)	229 (58.12%)	0 (0%)	165 (41.88%)
P	159 (92.98%)	0 (0%)	12 (7.02%)	157 (91.81%)	0 (0%)	14 (8.19%)
C	14 (2.75%)	6 (1.18%)	489 (96.07%)	20 (3.93%)	0 (0%)	489 (96.07%)
CIP	170 (32.95%)	26 (5.04%)	320 (62.02%)	180 (34.88%)	0 (0%)	336 (65.12%)
DA	59 (10.3%)	121 (21.12%)	393 (68.59%)	121 (21.12%)	79 (13.79%)	373 (65.1%)
E	332 (59.5%)	11 (1.97%)	215 (38.53%)	343 (61.47%)	37 (6.63%)	178 (31.9%)
GN	133 (27.2%)	9 (1.84%)	347 (70.96%)	190 (39%)	0 (0%)	299 (61%)
LZD	9 (1.63%)	0 (0%)	542 (98.37%)	5 (0.91%)	0 (0%)	546 (99.09%)
TEC	4 (0.7%)	0 (0%)	569 (99.3%)	N/A*	N/A*	N/A*

CLSI: Clinical & Laboratory Standards Institute; EUCAST: European Committee on Antimicrobial Susceptibility Testing; Ab: Antibiotic; R: Resistant isolates; I: Intermediate isolates; S: Susceptible isolates; FOX: Cefoxitin; P: Penicillin; C: Chloramphenicol; CIP: Ciprofloxacin; DA: Clindamycin; E: Erythromycin; GN: Gentamicin; LZD: Linezolid; TEC: Teicoplanin; N/A*: Not available, *i.e.*, breakpoint not supplied in the respective guideline.

Table 4 – ***Breakpoint interpretations for Staphylococcus aureus isolated from the Medical ward, for each of the antibiotics tested. CLSI and EUCAST interpretations presented side-by-side for comparison***

Ab	*Staphylococcus aureus*					
	CLSI Medical			EUCAST Medical		
	R# (R%)	*I# (I%)*	*S# (S%)*	*R# (R%)*	*I# (I%)*	*S# (S%)*
FOX	137 (47.9%)	0 (0%)	149 (52.1%)	137 (47.9%)	0 (0%)	149 (52.1%)
P	100 (89.29%)	0 (0%)	12 (10.71%)	98 (87.5%)	0 (0%)	14 (12.5%)
C	9 (2.49%)	2 (0.55%)	350 (96.95%)	11 (3.05%)	0 (0%)	350 (96.95%)
CIP	71 (19.61%)	22 (6.08%)	269 (74.31%)	80 (22.1%)	0 (0%)	282 (77.9%)
DA	40 (9.85%)	88 (21.67%)	278 (68.47%)	88 (21.67%)	57 (14.04%)	261 (64.29%)
E	207 (52.54%)	9 (2.28%)	178 (45.18%)	216 (54.82%)	27 (6.85%)	151 (38.32%)
GN	57 (16.91%)	7 (2.08%)	273 (81.01%)	174 (51.63%)	0 (0%)	163 (48.37%)
LZD	5 (1.28%)	0 (0%)	385 (98.72%)	2 (0.51%)	0 (0%)	388 (99.49%)
TEC	3 (0.74%)	0 (0%)	403 (99.26%)	N/A*	N/A*	N/A*

CLSI: Clinical & Laboratory Standards Institute; EUCAST: European Committee on Antimicrobial Susceptibility Testing; Ab: Antibiotic; R: Resistant isolates; I: Intermediate isolates; S: Susceptible isolates; FOX: Cefoxitin; P: Penicillin; C: Chloramphenicol; CIP: Ciprofloxacin; DA: Clindamycin; E: Erythromycin; GN: Gentamicin; LZD: Linezolid; TEC: Teicoplanin; N/A*: Not available, *i.e.*, breakpoint not supplied in the respective guideline.

Table 5 – ***Breakpoint interpretations for Staphylococcus aureus isolated from the ICU/Surgery wards, for each of the antibiotics tested. CLSI and EUCAST interpretations presented side-by-side for comparison***

Ab	*Staphylococcus aureus*					
	CLSI ICU/Surgery			EUCAST ICU/Surgery		
	R# (R%)	*I# (I%)*	*S# (S%)*	*R# (R%)*	*I# (I%)*	*S# (S%)*
FOX	92 (85.19%)	0 (0%)	16 (14.81%)	92 (85.19%)	0 (0%)	16 (14.81%)
P	59 (100%)	0 (0%)	0 (0%)	59 (100%)	0 (0%)	0 (0%)
C	5 (3.38%)	4 (2.7%)	139 (93.92%)	9 (6.08%)	0 (0%)	139 (93.92%)
CIP	99 (64.29%)	4 (2.6%)	51 (33.12%)	100 (64.94%)	0 (0%)	54 (35.06%)
DA	19 (11.38%)	33 (19.76%)	115 (68.86%)	33 (19.76%)	22 (13.17%)	112 (67.07%)
E	125 (76.22%)	2 (1.22%)	37 (22.56%)	127 (77.44%)	10 (6.1%)	27 (16.46%)
GN	76 (50%)	2 (1.32%)	74 (48.68%)	117 (76.97%)	0 (0%)	35 (23.03%)
LZD	4 (2.48%)	0 (0%)	157 (97.52%)	3 (1.86%)	0 (0%)	158 (98.14%)
TEC	1 (0.6%)	0 (0%)	166 (99.4%)	N/A*	N/A*	N/A*

ICU: Intensive Care Unit; CLSI: Clinical & Laboratory Standards Institute; EUCAST: European Committee on Antimicrobial Susceptibility Testing; Ab: Antibiotic; R: Resistant isolates; I: Intermediate isolates; S: Susceptible isolates; FOX: Cefoxitin; P: Penicillin; C: Chloramphenicol; CIP: Ciprofloxacin; DA: Clindamycin; E: Erythromycin; GN: Gentamicin; LZD: Linezolid; TEC: Teicoplanin; N/A*: Not available, *i.e.*, breakpoint not supplied in the respective guideline.

Resistance to macrolides is high (around 60%). Due to their bacteriostatic nature, macrolides are not the treatment of choice.

Clindamycin resistance differed significantly according to the breakpoints used, although the sum of resistant and intermediate samples is constant. The EUCAST guidelines interpret more isolates as being resistant (10% *vs.* 21% – n=59 *vs.* 121, p<0.001). Resistance to Clindamycin was tested using the "*D*-test" technique to check for inducible resistance.

Chloramphenicol, Linezolid and Teicoplanin resistance are all low. There are no interpretation breakpoints for Teicoplanin. Linezolid resistant samples need to be confirmed through quantitative MIC studies.

We already mentioned the differences in MRSA isolates between wards.

It would be interesting to know if, between MRSA isolates, resistance to Ciprofloxacin and Gentamicin covariate, both in Medical ward and ICU/Surgery ward. Thus, a conclusion as to the community-acquired and hospital/healthcare-associated isolates cannot be taken at this moment [22, 23]. It should be explored further in future work.

While Vancomycin susceptibility was originally tested, we decided to exclude it from the analysis due to newer recommendations to perform quantitative (MIC) studies to determine possible resistance. All isolates found to be intermediate/resistant to Vancomycin must be tested by a Reference Laboratory, due to the fear of possible spread of Vancomycin-intermediate *S. aureus*/Vancomycin-resistant *S. aureus* (VISA/VRSA) strains.

Klebsiella spp.

ICU/Surgery isolates accounted for 35% (n=163) of total isolates that fit the inclusion criteria.

The antibiogram profiles show a high resistance in many important classes of antibiotics like beta-lactams [extended-spectrum β-lactamase (ESBL) and Carbapenemase producers], quinolones and aminoglycosides.

Overall resistance to antibiotics is presented in Figure 6 and accompanying Table 6.

The difference between the resistance patterns in the ICU/Surgical wards and the Medical wards are stark in Figure 7. We will discuss some general patterns as well as the specific differences that emerge between the wards, as shown in Tables 7 and 8.

Resistance to amino-beta-lactams is high, over 50% in both ICU/Surgery and Medical wards (Ampicillin resistance is considered natural for these isolates). ESBL isolates are present in about 40% of isolates (21% in the Medical wards – 70% in the ICU/Surgery wards, p<0.001).

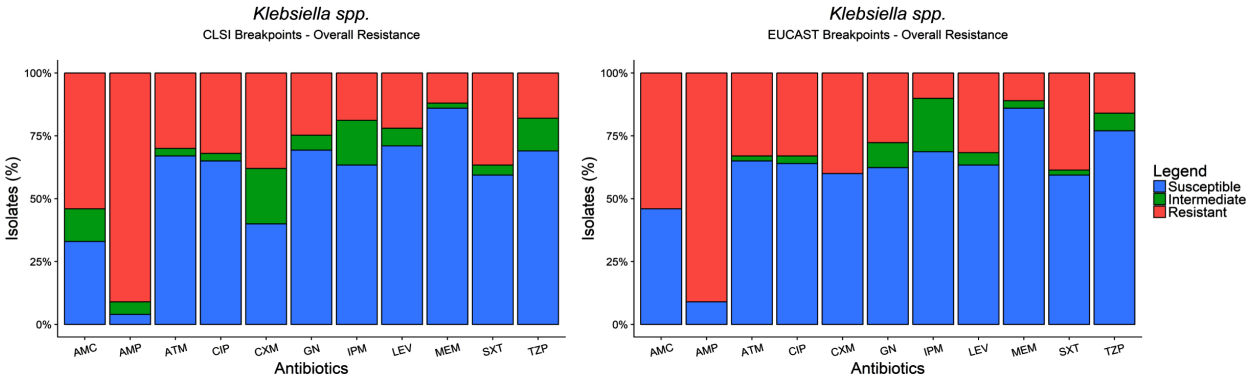

Figure 6 – ***Barplot showing the overall resistance of Klebsiella pneumoniae, interpreting antibiograms as per the CLSI and EUCAST breakpoints respectively. CLSI: Clinical & Laboratory Standards Institute; EUCAST: European Committee on Antimicrobial Susceptibility Testing; AMC: Amoxicillin; AMP: Ampicillin; ATM: Aztreonam; CIP: Ciprofloxacin; CXM: Cefuroxime; GN: Gentamicin; IPM: Imipenem; LEV: Levofloxacin; MEM: Meropenem; SXT: Trimethoprim–Sulfamethoxazole; TZP: Piperacillin–Tazobactam.***

Table 6 – ***Breakpoint interpretations for Klebsiella spp. for each of the antibiotics tested. CLSI and EUCAST interpretations side by side for comparison***

	***Klebsiella* spp.**					
Ab	**CLSI Overview**			**EUCAST Overview**		
	R# (R%)	***I# (I%)***	***S# (S%)***	***R# (R%)***	***I# (I%)***	***S# (S%)***
AMP	417 (90.85%)	22 (4.79%)	20 (4.36%)	417 (90.85%)	0 (0%)	42 (9.15%)
AMC	161 (53.85%)	38 (12.71%)	100 (33.44%)	206 (68.9%)	0 (0%)	93 (31.1%)
ATM	116 (29.52%)	13 (3.31%)	264 (67.18%)	129 (32.82%)	8 (2.04%)	256 (65.14%)
CXM	143 (38.24%)	83 (22.19%)	148 (39.57%)	151 (40.37%)	0 (0%)	223 (59.63%)
GN	90 (24.79%)	20 (5.51%)	253 (69.7%)	101 (27.82%)	35 (9.64%)	227 (62.53%)
CIP	127 (34.05%)	4 (1.07%)	242 (64.88%)	123 (32.98%)	12 (3.22%)	238 (63.81%)
LEV	89 (21.87%)	29 (7.13%)	289 (71.01%)	129 (31.7%)	19 (4.67%)	259 (63.64%)
SXT	130 (36.72%)	13 (3.67%)	211 (59.6%)	137 (38.7%)	6 (1.69%)	211 (59.6%)
IPM	76 (18.81%)	71 (17.57%)	257 (63.61%)	42 (10.4%)	86 (21.29%)	276 (68.32%)
MEM	49 (11.92%)	10 (2.43%)	352 (85.64%)	44 (10.71%)	12 (2.92%)	355 (86.37%)
TZP	66 (18.33%)	47 (13.06%)	247 (68.61%)	56 (15.56%)	26 (7.22%)	278 (77.22%)

CLSI: Clinical & Laboratory Standards Institute; EUCAST: European Committee on Antimicrobial Susceptibility Testing; Ab: Antibiotic; R: Resistant isolates; I: Intermediate isolates; S: Susceptible isolates; AMP: Ampicillin; AMC: Amoxicillin; ATM: Aztreonam; CXM: Cefuroxime; GN: Gentamicin; CIP: Ciprofloxacin; LEV: Levofloxacin; SXT: Trimethoprim–Sulfamethoxazole; IPM: Imipenem; MEM: Meropenem; TZP: Piperacillin–Tazobactam.

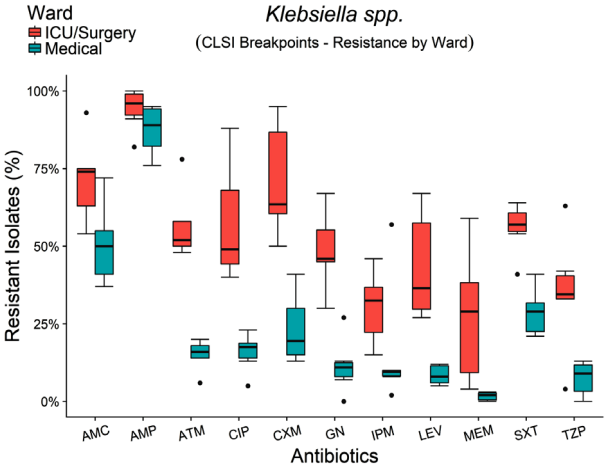

Figure 7 – ***Boxplots showing the variability of recorded resistance between isolates of Klebsiella spp. received from the ICU/Surgery and the Medical ward. ICU: Intensive Care Unit; CLSI: Clinical & Laboratory Standards Institute; AMC: Amoxicillin; AMP: Ampicillin; ATM: Aztreonam; CIP: Ciprofloxacin; CXM: Cefuroxime; GN: Gentamicin; IPM: Imipenem; LEV: Levofloxacin; MEM: Meropenem; SXT: Trimethoprim–Sulfamethoxazole; TZP: Piperacillin–Tazobactam.***

Aztreonam, a monobactam seldom used in Romania also has an overall resistance rate of about 31%, with high differences between Medical and ICU/Surgery ward (15% *vs.* 57%, $p<0.001$), but no differences between the diagnostic criteria proposed by EUCAST and CLSI.

The combination of ureidopenicillin Piperacillin and the beta-lactamase inhibitor Tazobactam has the lowest levels of resistance of non-Carbapenem beta-lactams, accounting for <10% resistance in the Medical wards, 33% in the Surgery wards ($p<0.01$), and an overall resistance of 15% (EUCAST) – 18% (CLSI) of overall isolates.

A potentially clinically important difference arises when comparing EUCAST and CLSI breakpoint definitions for Carbapenems. Out of the two Carbapenems tested, Meropenem has slightly lower resistance rates (~11% of tested). Meropenem resistance seems low and stable in the Medical ward. This is not the case for the ICU/Surgery ward, where resistance varies more (even more than 50%). The median (overall) level of Meropenem was around 30% for the ICU/Surgery ward. Imipenem resistance is higher and follows the same peculiarities. Applying the EUCAST breakpoints, fewer isolates would be classified as Resistant to Imipenem (10% EUCAST – 18% CLSI, $p<0.01$). Recently, the CLSI breakpoints have been modified to harmonize the susceptibility breakpoints.

Table 7 – ***Breakpoint interpretations for Klebsiella spp. isolated from the Medical ward, for each of the antibiotics tested. CLSI and EUCAST interpretations presented side-by-side for comparison***

	***Klebsiella* spp.**					
Ab	**CLSI Medical**			**EUCAST Medical**		
	R# (R%)	***I# (I%)***	***S# (S%)***	***R# (R%)***	***I# (I%)***	***S# (S%)***
AMP	265 (88.93%)	16 (5.37%)	17 (5.7%)	265 (88.93%)	0 (0%)	33 (11.07%)
AMC	89 (44.95%)	32 (16.16%)	77 (38.89%)	126 (63.64%)	0 (0%)	72 (36.36%)
ATM	41 (15.59%)	8 (3.04%)	214 (81.37%)	49 (18.63%)	4 (1.52%)	210 (79.85%)
CXM	52 (21.4%)	71 (29.22%)	120 (49.38%)	59 (24.28%)	0 (0%)	184 (75.72%)
GN	27 (11.64%)	14 (6.03%)	191 (82.33%)	34 (14.66%)	27 (11.64%)	171 (73.71%)
CIP	46 (19.17%)	3 (1.25%)	191 (79.58%)	42 (17.5%)	11 (4.58%)	187 (77.92%)
LEV	24 (9.13%)	11 (4.18%)	228 (86.69%)	40 (15.21%)	15 (5.7%)	208 (79.09%)
SXT	67 (28.27%)	10 (4.22%)	160 (67.51%)	72 (30.38%)	5 (2.11%)	160 (67.51%)
IPM	32 (12.21%)	48 (18.32%)	182 (69.47%)	10 (3.82%)	54 (20.61%)	198 (75.57%)
MEM	6 (2.27%)	4 (1.52%)	254 (96.21%)	3 (1.14%)	6 (2.27%)	255 (96.59%)
TZP	20 (8.58%)	19 (8.15%)	194 (83.26%)	14 (6.01%)	10 (4.29%)	209 (89.7%)

CLSI: Clinical & Laboratory Standards Institute; EUCAST: European Committee on Antimicrobial Susceptibility Testing; Ab: Antibiotic; R: Resistant isolates; I: Intermediate isolates; S: Susceptible isolates; AMP: Ampicillin; AMC: Amoxicillin; ATM: Aztreonam; CXM: Cefuroxime; GN: Gentamicin; CIP: Ciprofloxacin; LEV: Levofloxacin; SXT: Trimethoprim–Sulfamethoxazole; IPM: Imipenem; MEM: Meropenem; TZP: Piperacillin–Tazobactam.

Table 8 – ***Breakpoint interpretations for Klebsiella spp. isolated from the ICU/Surgery wards, for each of the antibiotics tested. CLSI and EUCAST interpretations presented side-by-side for comparison***

	***Klebsiella* spp.**					
Ab	**CLSI ICU/Surgery**			**EUCAST ICU/Surgery**		
	R# (R%)	***I# (I%)***	***S# (S%)***	***R# (R%)***	***I# (I%)***	***S# (S%)***
AMP	152 (94.41%)	6 (3.73%)	3 (1.86%)	152 (94.41%)	0 (0%)	9 (5.59%)
AMC	72 (71.29%)	6 (5.94%)	23 (22.77%)	80 (79.21%)	0 (0%)	21 (20.79%)
ATM	75 (57.69%)	5 (3.85%)	50 (38.46%)	80 (61.54%)	4 (3.08%)	46 (35.38%)
CXM	91 (69.47%)	12 (9.16%)	28 (21.37%)	92 (70.23%)	0 (0%)	39 (29.77%)
GN	63 (48.09%)	6 (4.58%)	62 (47.33%)	67 (51.15%)	8 (6.11%)	56 (42.75%)
CIP	81 (60.9%)	1 (0.75%)	51 (38.35%)	81 (60.9%)	1 (0.75%)	51 (38.35%)
LEV	65 (45.14%)	18 (12.5%)	61 (42.36%)	89 (61.81%)	4 (2.78%)	51 (35.42%)
SXT	63 (53.85%)	3 (2.56%)	51 (43.59%)	65 (55.56%)	1 (0.85%)	51 (43.59%)
IPM	44 (30.99%)	23 (16.2%)	75 (52.82%)	32 (22.54%)	32 (22.54%)	78 (54.93%)
MEM	43 (29.25%)	6 (4.08%)	98 (66.67%)	41 (27.89%)	6 (4.08%)	100 (68.03%)
TZP	46 (36.22%)	28 (22.05%)	53 (41.73%)	42 (33.07%)	16 (12.6%)	69 (54.33%)

ICU: Intensive Care Unit; CLSI: Clinical & Laboratory Standards Institute; EUCAST: European Committee on Antimicrobial Susceptibility Testing; Ab: Antibiotic; R: Resistant isolates; I: Intermediate isolates; S: Susceptible isolates; AMP: Ampicillin; AMC: Amoxicillin; ATM: Aztreonam; CXM: Cefuroxime; GN: Gentamicin; CIP: Ciprofloxacin; LEV: Levofloxacin; SXT: Trimethoprim–Sulfamethoxazole; IPM: Imipenem; MEM: Meropenem; TZP: Piperacillin–Tazobactam.

Quinolone breakpoints did not influence resistance levels for Ciprofloxacin, with about one third of isolates being resistant. Differences between Medical and non-Medical wards were significant (20% *vs.* 60%, $p<0.001$). Levofloxacin resistance shows about 10% difference when comparing breakpoints (21% EUCAST *vs.* 31% CLSI, $p<0.01$), following the same kind of Medical-ICU/Surgery pattern ($p<0.001$).

Aminoglycoside resistance is found in about 25% of isolates, but can reach 50% in the ICU/Surgery ward ($p<0.001$).

Acinetobacter baumannii

The redoubtable pathogen *Acinetobacter baumannii* was most frequently encountered in the ICU/Surgery ward.

In fact, *A. baumannii* was the only pathogen that was consistently isolated more in the ICU/Surgery departments than in the Medical wards.

Out of the 529 isolates that belong to adult inpatients 341 (64%) were found in the ICU/Surgery ward. Of note, EUCAST guidelines have not set any breakpoints for interpreting disk diffusion susceptibility data for cephalosporins, carboxy- and ureido-penicillins (Figures 8 and 9; Tables 9–11).

Nor are there clinical breakpoints for polymyxins.

Recently, the *E*-test was discouraged as a method to be used, recommending classic MIC methods [24, 25].

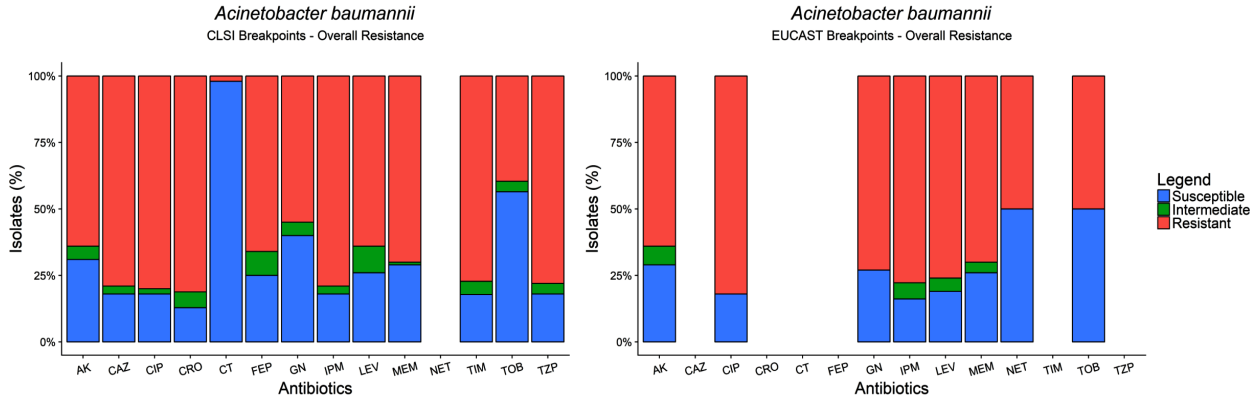

Figure 8 – ***Barplot showing the overall resistance of Acinetobacter baumannii, interpreting antibiograms as per the CLSI and EUCAST breakpoints respectively. CLSI: Clinical & Laboratory Standards Institute; EUCAST: European Committee on Antimicrobial Susceptibility Testing; AK: Amikacin; CAZ: Ceftazidime; CIP: Ciprofloxacin; CRO: Ceftriaxone; CT: Colistin; FEP: Cefepime; GN: Gentamicin; IPM: Imipenem; LEV: Levofloxacin; MEM: Meropenem; NET: Netilmicin; TIM: Ticarcillin–Clavulanic acid; TOB: Tobramycin; TZP: Piperacillin–Tazobactam.***

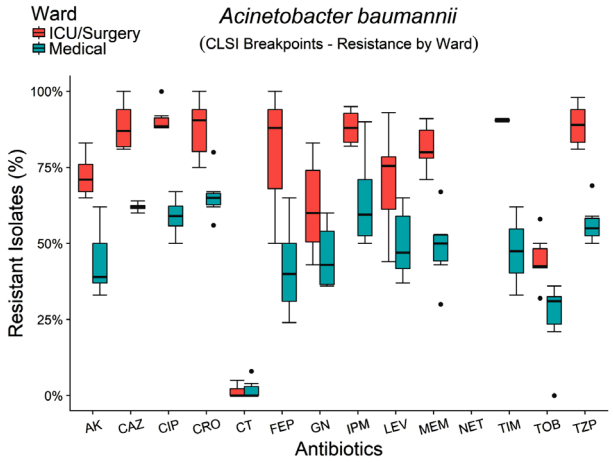

Figure 9 – ***Boxplots showing the variability of recorded resistance between isolates of Acinetobacter baumannii received from the ICU/Surgery and the Medical ward. ICU: Intensive Care Unit; CLSI: Clinical & Laboratory Standards Institute; AK: Amikacin; CAZ: Ceftazidime; CIP: Ciprofloxacin; CRO: Ceftriaxone; CT: Colistin; FEP: Cefepime; GN: Gentamicin; IPM: Imipenem; LEV: Levofloxacin; MEM: Meropenem; NET: Netilmicin; TIM: Ticarcillin–Clavulanic acid; TOB: Tobramycin; TZP: Piperacillin–Tazobactam.***

There are also consistent differences in the susceptibility profile, with Medical ward isolates having fewer overall resistant strains. While this may seem favorable, the difference is doubtful to be able to impact clinical practice due to the high resistance rates in all antibiotic classes with the exception of polymyxins (Figure 9).

Overall beta-lactam resistance is high, particularly in third generation cephalosporins Ceftriaxone (81%, *n*=322) and Ceftazidime (81.6%, *n*=266). Cefepime, a fourth generation cephalosporin has a slightly milder resistance profile, under the reservation that just over a third of isolates were tested (66%, *n*=173). Of note, on the Medical wards, resistance to Cefepime in addition to intermediate isolates would yield that roughly 75% (*n*=196) of *A. baumannii* are non-susceptible. Carboxy- and ureidopenicillins have similar levels of resistance – the more often tested Piperacillin–Tazobactam (*n*=361) had an overall resistance of 78%.

Carbapenem resistance is worryingly high, with rates jumping to over 50% both in the Medical ward, as well as in the ICU/Surgery ward. Differences are small, yet statistically significant ($p<0.05$).

Table 9 – ***Breakpoint interpretations for Acinetobacter baumannii for each of the antibiotics tested. CLSI and EUCAST interpretations side by side for comparison***

Ab	*Acinetobacter baumannii*					
	CLSI Overview			EUCAST Overview		
	R# (R%)	*I# (I%)*	*S# (S%)*	*R# (R%)*	*I# (I%)*	*S# (S%)*
AK	213 (63.77%)	17 (5.09%)	104 (31.14%)	213 (63.77%)	23 (6.89%)	98 (29.34%)
GN	200 (55.1%)	19 (5.23%)	144 (39.67%)	264 (72.73%)	0 (0%)	99 (27.27%)
TOB	160 (39.6%)	15 (3.71%)	229 (56.68%)	201 (49.75%)	0 (0%)	203 (50.25%)
NET	N/A*	N/A*	N/A*	212 (50.24%)	0 (0%)	210 (49.76%)
CRO	322 (81.73%)	22 (5.58%)	50 (12.69%)	N/A*	N/A*	N/A*
CAZ	266 (81.6%)	0 (0%)	60 (18.4%)	N/A*	N/A*	N/A*
FEP	173 (66.03%)	23 (8.78%)	66 (25.19%)	N/A*	N/A*	N/A*
TZP	282 (78.12%)	14 (3.88%)	65 (18.01%)	N/A*	N/A*	N/A*
TIM	83 (77.57%)	5 (4.67%)	19 (17.76%)	N/A*	N/A*	N/A*
CIP	298 (80.11%)	6 (1.61%)	68 (18.28%)	304 (81.72%)	0 (0%)	68 (18.28%)
LEV	274 (63.57%)	43 (9.98%)	114 (26.45%)	328 (76.1%)	21 (4.87%)	82 (19.03%)

Ab	*Acinetobacter baumannii*					
	CLSI Overview			EUCAST Overview		
	R# (R%)	*I# (I%)*	*S# (S%)*	*R# (R%)*	*I# (I%)*	*S# (S%)*
IPM	302 (78.85%)	13 (3.39%)	68 (17.75%)	296 (77.28%)	24 (6.27%)	63 (16.45%)
MEM	283 (69.88%)	6 (1.48%)	116 (28.64%)	283 (69.88%)	15 (3.7%)	107 (26.42%)
CT	8 (1.62%)	0 (0%)	486 (98.38%)	N/A*	N/A*	N/A*

CLSI: Clinical & Laboratory Standards Institute; EUCAST: European Committee on Antimicrobial Susceptibility Testing; Ab: Antibiotic; R: Resistant isolates; I: Intermediate isolates; S: Susceptible isolates; AK: Amikacin; GN: Gentamicin; TOB: Tobramycin; NET: Netilmicin; CRO: Ceftriaxone; CAZ: Ceftazidime; FEP: Cefepime; TZP: Piperacillin–Tazobactam; TIM: Ticarcillin–Clavulanic acid; CIP: Ciprofloxacin; LEV: Levofloxacin; IPM: Imipenem; MEM: Meropenem; CT: Colistin; N/A*: Not available, *i.e.*, breakpoint not supplied in the respective guideline.

Table 10 – ***Breakpoint interpretations for Acinetobacter baumannii isolated from the Medical ward, for each of the antibiotics tested. CLSI and EUCAST interpretations presented side-by-side for comparison***

Ab	*Acinetobacter baumannii*					
	CLSI Medical			EUCAST Medical		
	R# (R%)	*I# (I%)*	*S# (S%)*	*R# (R%)*	*I# (I%)*	*S# (S%)*
AK	40 (40.82%)	7 (7.14%)	51 (52.04%)	40 (40.82%)	8 (8.16%)	50 (51.02%)
GN	52 (44.83%)	1 (0.86%)	63 (54.31%)	68 (58.62%)	0 (0%)	48 (41.38%)
TOB	36 (27.07%)	7 (5.26%)	90 (67.67%)	49 (36.84%)	0 (0%)	84 (63.16%)
NET	N/A*	N/A*	N/A*	48 (35.04%)	0 (0%)	89 (64.96%)
CRO	83 (66.94%)	10 (8.06%)	31 (25%)	N/A*	N/A*	N/A*
CAZ	76 (66.67%)	0 (0%)	38 (33.33%)	N/A*	N/A*	N/A*
FEP	35 (43.75%)	9 (11.25%)	36 (45%)	N/A*	N/A*	N/A*
TZP	64 (56.64%)	7 (6.19%)	42 (37.17%)	N/A*	N/A*	N/A*
TIM	12 (41.38%)	4 (13.79%)	13 (44.83%)	N/A*	N/A*	N/A*
CIP	70 (58.82%)	3 (2.52%)	46 (38.66%)	73 (61.34%)	0 (0%)	46 (38.66%)
LEV	67 (47.86%)	12 (8.57%)	61 (43.57%)	83 (59.29%)	7 (5%)	50 (35.71%)
IPM	72 (60%)	6 (5%)	42 (35%)	67 (55.83%)	12 (10%)	41 (34.17%)
MEM	62 (47.33%)	3 (2.29%)	66 (50.38%)	62 (47.33%)	6 (4.58%)	63 (48.09%)
CT	3 (1.86%)	0 (0%)	158 (98.14%)	N/A*	N/A*	N/A*

CLSI: Clinical & Laboratory Standards Institute; EUCAST: European Committee on Antimicrobial Susceptibility Testing; Ab: Antibiotic; R: Resistant isolates; I: Intermediate isolates; S: Susceptible isolates; AK: Amikacin; GN: Gentamicin; TOB: Tobramycin; NET: Netilmicin; CRO: Ceftriaxone; CAZ: Ceftazidime; FEP: Cefepime; TZP: Piperacillin–Tazobactam; TIM: Ticarcillin–Clavulanic acid; CIP: Ciprofloxacin; LEV: Levofloxacin; IPM: Imipenem; MEM: Meropenem; CT: Colistin; N/A*: Not available, *i.e.*, breakpoint not supplied in the respective guideline.

Table 11 – ***Breakpoint interpretations for Acinetobacter baumannii isolated from the ICU/Surgery wards, for each of the antibiotics tested. CLSI and EUCAST interpretations presented side-by-side for comparison***

Ab	*Acinetobacter baumannii*					
	CLSI ICU/Surgery			EUCAST ICU/Surgery		
	R# (R%)	*I# (I%)*	*S# (S%)*	*R# (R%)*	*I# (I%)*	*S# (S%)*
AK	173 (73.31%)	10 (4.24%)	53 (22.46%)	173 (73.31%)	15 (6.36%)	48 (20.34%)
GN	148 (59.92%)	18 (7.29%)	81 (32.79%)	196 (79.35%)	0 (0%)	51 (20.65%)
TOB	124 (45.76%)	8 (2.95%)	139 (51.29%)	152 (56.09%)	0 (0%)	119 (43.91%)
NET	N/A*	N/A*	N/A*	164 (57.34%)	0 (0%)	122 (42.66%)
CRO	239 (88.52%)	12 (4.44%)	19 (7.04%)	N/A*	N/A*	N/A*
CAZ	185 (87.26%)	5 (2.36%)	22 (10.38%)	N/A*	N/A*	N/A*
FEP	138 (75.82%)	14 (7.69%)	30 (16.48%)	N/A*	N/A*	N/A*
TZP	218 (87.9%)	7 (2.82%)	23 (9.27%)	N/A*	N/A*	N/A*
TIM	71 (91.03%)	1 (1.28%)	6 (7.69%)	N/A*	N/A*	N/A*
CIP	228 (90.12%)	3 (1.19%)	22 (8.7%)	231 (91.3%)	0 (0%)	22 (8.7%)
LEV	207 (71.13%)	31 (10.65%)	53 (18.21%)	245 (84.19%)	14 (4.81%)	32 (11%)
IPM	230 (87.45%)	7 (2.66%)	26 (9.89%)	229 (87.07%)	12 (4.56%)	22 (8.37%)
MEM	221 (80.66%)	3 (1.09%)	50 (18.25%)	221 (80.66%)	9 (3.28%)	44 (16.06%)
CT	5 (1.5%)	0 (0%)	328 (98.5%)	N/A*	N/A*	N/A*

ICU: Intensive Care Unit; CLSI: Clinical & Laboratory Standards Institute; EUCAST: European Committee on Antimicrobial Susceptibility Testing; Ab: Antibiotic; R: Resistant isolates; I: Intermediate isolates; S: Susceptible isolates; AK: Amikacin; GN: Gentamicin; TOB: Tobramycin; NET: Netilmicin; CRO: Ceftriaxone; CAZ: Ceftazidime; FEP: Cefepime; TZP: Piperacillin–Tazobactam; TIM: Ticarcillin–Clavulanic acid; CIP: Ciprofloxacin; LEV: Levofloxacin; IPM: Imipenem; MEM: Meropenem; CT: Colistin; N/A*: Not available, *i.e.*, breakpoint not supplied in the respective guideline.

Pseudomonas aeruginosa

Pseudomonas aeruginosa was the most frequent pathogen cultured from the respiratory samples of our study population of adult inpatients, with 2065 isolates between 2010 and 2015. Isolates from the ICU/Surgery ward accounted for 30% (*n*=401), out of the total 1296.

Two significant differences arise from the comparison of CLSI and EUCAST breakpoint interpretation data (Figure 10, Table 12). First, while Ticarcillin–Clavulanic acid breakpoints yield about the same number of Resistant isolates (*n*=92 *vs.* 98, 35.8% *vs.* 38.1%, CLSI *vs.* EUCAST), CLSI guidelines classify an additional 44 isolates (17.12%) as Intermediate ($p<0.01$). Secondly, CLSI guidelines classify fewer isolates as resistant to Piperacillin (14.2% *vs.* 23.8%, *n*=140 *vs.* 234, CLSI *vs.* EUCAST), but introduces a considerate number of them into the "Intermediate" category (19.33%, *n*=190) so that, there is no statistical differences (Tables 13 and 14).

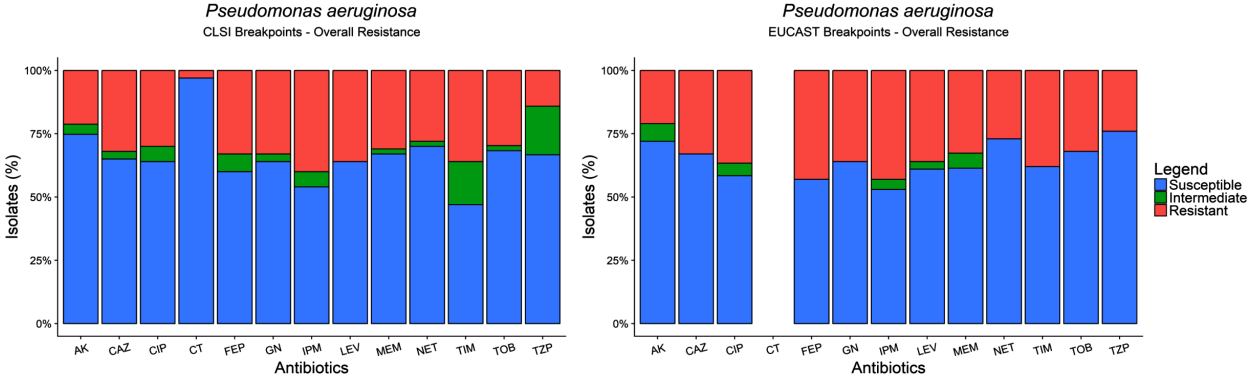

Figure 10 – ***Barplot showing the overall resistance of Pseudomonas aeruginosa, interpreting antibiograms as per the CLSI and EUCAST breakpoints respectively. CLSI: Clinical & Laboratory Standards Institute; EUCAST: European Committee on Antimicrobial Susceptibility Testing; AK: Amikacin; CAZ: Ceftazidime; CIP: Ciprofloxacin; CT: Colistin; FEP: Cefepime; GN: Gentamicin; IPM: Imipenem; LEV: Levofloxacin; MEM: Meropenem; NET: Netilmicin; TIM: Ticarcillin–Clavulanic acid; TOB: Tobramycin; TZP: Piperacillin–Tazobactam.***

Table 12 – ***Breakpoint interpretations for Acinetobacter baumannii for each of the antibiotics tested. CLSI and EUCAST interpretations side by side for comparison***

Pseudomonas aeruginosa						
Ab	**CLSI Overview**			**EUCAST Overview**		
	R# (R%)	***I# (I%)***	***S# (S%)***	***R# (R%)***	***I# (I%)***	***S# (S%)***
AK	194 (21.06%)	41 (4.45%)	686 (74.48%)	194 (21.06%)	65 (7.06%)	662 (71.88%)
GN	316 (33.47%)	24 (2.54%)	604 (63.98%)	340 (36.02%)	0 (0%)	604 (63.98%)
TOB	322 (29.51%)	18 (1.65%)	751 (68.84%)	340 (31.16%)	0 (0%)	751 (68.84%)
NET	320 (27.97%)	21 (1.84%)	803 (70.19%)	312 (27.27%)	0 (0%)	832 (72.73%)
CIP	278 (30.28%)	51 (5.56%)	589 (64.16%)	336 (36.56%)	42 (4.57%)	541 (58.87%)
LEV	353 (31.52%)	48 (4.29%)	719 (64.2%)	401 (35.8%)	38 (3.39%)	681 (60.8%)
MEM	340 (30.94%)	24 (2.18%)	735 (66.88%)	360 (32.76%)	62 (5.64%)	677 (61.6%)
IPM	408 (39.69%)	64 (6.23%)	556 (54.09%)	439 (42.7%)	46 (4.47%)	543 (52.82%)
CT	42 (3.41%)	0 (0%)	1191 (96.59%)	N/A*	N/A*	N/A*
TZP	140 (14.24%)	190 (19.33%)	653 (66.43%)	234 (23.8%)	0 (0%)	749 (76.2%)
TIM	92 (35.8%)	44 (17.12%)	121 (47.08%)	98 (38.13%)	0 (0%)	159 (61.87%)
CAZ	348 (31.84%)	37 (3.39%)	708 (64.78%)	363 (33.21%)	0 (0%)	730 (66.79%)
FEP	237 (33.01%)	51 (7.1%)	430 (59.89%)	309 (43.04%)	0 (0%)	409 (56.96%)

CLSI: Clinical & Laboratory Standards Institute; EUCAST: European Committee on Antimicrobial Susceptibility Testing; Ab: Antibiotic; R: Resistant isolates; I: Intermediate isolates; S: Susceptible isolates; AK: Amikacin; GN: Gentamicin; TOB: Tobramycin; NET: Netilmicin; CIP: Ciprofloxacin; LEV: Levofloxacin; MEM: Meropenem; IPM: Imipenem; CT: Colistin; TZP: Piperacillin–Tazobactam; TIM: Ticarcillin–Clavulanic acid; CAZ: Ceftazidime; FEP: Cefepime; N/A*: Not available, *i.e.*, breakpoint not supplied in the respective guideline.

Table 13 – ***Breakpoint interpretations for Pseudomonas aeruginosa isolated from the Medical ward, for each of the antibiotics tested. CLSI and EUCAST interpretations presented side-by-side for comparison***

Pseudomonas aeruginosa						
Ab	**CLSI Medical**			**EUCAST Medical**		
	R# (R%)	***I# (I%)***	***S# (S%)***	***R# (R%)***	***I# (I%)***	***S# (S%)***
AK	61 (9.5%)	17 (2.65%)	564 (87.85%)	61 (9.5%)	31 (4.83%)	550 (85.67%)
GN	127 (19.3%)	17 (2.58%)	514 (78.12%)	144 (21.88%)	0 (0%)	514 (78.12%)
TOB	122 (15.6%)	9 (1.15%)	651 (83.25%)	141 (18.03%)	0 (0%)	641 (81.97%)
NET	120 (14.71%)	20 (2.45%)	676 (82.84%)	116 (14.22%)	0 (0%)	700 (85.78%)
CIP	108 (16.85%)	36 (5.62%)	497 (77.54%)	149 (23.24%)	30 (4.68%)	462 (72.07%)
LEV	180 (22.96%)	0 (0%)	604 (77.04%)	180 (22.96%)	30 (3.83%)	574 (73.21%)
MEM	133 (17.21%)	13 (1.68%)	627 (81.11%)	143 (18.5%)	47 (6.08%)	583 (75.42%)

Ab	*Pseudomonas aeruginosa*					
	CLSI Medical			EUCAST Medical		
	R# (R%)	*I# (I%)*	*S# (S%)*	*R# (R%)*	*I# (I%)*	*S# (S%)*
IPM	197 (27.13%)	45 (6.2%)	484 (66.67%)	218 (30.03%)	33 (4.55%)	475 (65.43%)
CT	24 (2.83%)	0 (0%)	823 (97.17%)	N/A*	N/A*	N/A*
TZP	67 (9.64%)	92 (13.24%)	536 (77.12%)	105 (15.11%)	0 (0%)	590 (84.89%)
TIM	27 (16.46%)	34 (20.73%)	103 (62.8%)	31 (18.9%)	0 (0%)	133 (81.1%)
CAZ	146 (19.01%)	28 (3.65%)	594 (77.34%)	159 (20.7%)	0 (0%)	609 (79.3%)
FEP	98 (19.41%)	34 (6.73%)	373 (73.86%)	148 (29.31%)	0 (0%)	357 (70.69%)

CLSI: Clinical & Laboratory Standards Institute; EUCAST: European Committee on Antimicrobial Susceptibility Testing; Ab: Antibiotic; R: Resistant isolates; I: Intermediate isolates; S: Susceptible isolates; AK: Amikacin; GN: Gentamicin; TOB: Tobramycin; NET: Netilmicin; CIP: Ciprofloxacin; LEV: Levofloxacin; MEM: Meropenem; IPM: Imipenem; CT: Colistin; TZP: Piperacillin–Tazobactam; TIM: Ticarcillin–Clavulanic acid; CAZ: Ceftazidime; FEP: Cefepime; N/A*: Not available, *i.e.*, breakpoint not supplied in the respective guideline.

Table 14 – ***Breakpoint interpretations for Pseudomonas aeruginosa isolated from the ICU/Surgery wards, for each of the antibiotics tested. CLSI and EUCAST interpretations presented side-by-side for comparison***

Ab	*Pseudomonas aeruginosa*					
	CLSI ICU/Surgery			EUCAST ICU/Surgery		
	R# (R%)	*I# (I%)*	*S# (S%)*	*R# (R%)*	*I# (I%)*	*S# (S%)*
AK	133 (47.67%)	24 (8.6%)	122 (43.73%)	133 (47.67%)	34 (12.19%)	112 (40.14%)
GN	189 (66.08%)	7 (2.45%)	90 (31.47%)	196 (68.53%)	0 (0%)	90 (31.47%)
TOB	200 (64.72%)	9 (2.91%)	100 (32.36%)	209 (67.64%)	0 (0%)	100 (32.36%)
NET	200 (60.98%)	1 (0.3%)	127 (38.72%)	196 (59.76%)	0 (0%)	132 (40.24%)
CIP	170 (61.15%)	15 (5.4%)	93 (33.45%)	187 (67.27%)	12 (4.32%)	79 (28.42%)
LEV	207 (61.61%)	14 (4.17%)	115 (34.23%)	221 (65.77%)	8 (2.38%)	107 (31.85%)
MEM	207 (63.5%)	11 (3.37%)	108 (33.13%)	217 (66.56%)	15 (4.6%)	94 (28.83%)
IPM	211 (69.87%)	19 (6.29%)	72 (23.84%)	221 (73.18%)	13 (4.3%)	68 (22.52%)
CT	18 (4.66%)	0 (0%)	368 (95.34%)	N/A*	N/A*	N/A*
TZP	73 (25.35%)	98 (34.03%)	117 (40.63%)	129 (44.79%)	0 (0%)	159 (55.21%)
TIM	202 (62.15%)	9 (2.77%)	114 (35.08%)	204 (62.77%)	0 (0%)	121 (37.23%)
CAZ	139 (65.26%)	17 (7.98%)	57 (26.76%)	161 (75.59%)	0 (0%)	52 (24.41%)
FEP	65 (69.89%)	10 (10.75%)	18 (19.35%)	67 (72.04%)	0 (0%)	26 (27.96%)

ICU: Intensive Care Unit; CLSI: Clinical & Laboratory Standards Institute; EUCAST: European Committee on Antimicrobial Susceptibility Testing; Ab: Antibiotic; R: Resistant isolates; I: Intermediate isolates; S: Susceptible isolates; AK: Amikacin; GN: Gentamicin; TOB: Tobramycin; NET: Netilmicin; CIP: Ciprofloxacin; LEV: Levofloxacin; MEM: Meropenem; IPM: Imipenem; CT: Colistin; TZP: Piperacillin–Tazobactam; TIM: Ticarcillin–Clavulanic acid; CAZ: Ceftazidime; FEP: Cefepime; N/A*: Not available, *i.e.*, breakpoint not supplied in the respective guideline.

Differences between ICU/Surgery and Medical wards is highly important for most of the antibiotics tested ($p<0.05$ for Piperacillin–Tazobactam, $p<0.001$ for all the others), with the exception of Colistin. Rates of resistance in the ICU/Surgery ward were roughly two fold higher than in the Medical wards (Figure 11).

Of the beta-lactams tested, Piperacillin–Tazobactam had the lowest levels of resistance. Applying the CLSI guideline, this led to a significant drop in Resistance levels even in ICU/Surgery samples. It should be noted though, that a lot more isolates would be classified as Intermediate.

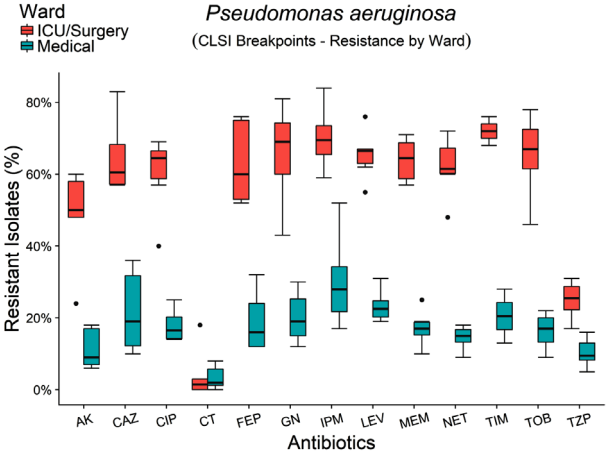

Figure 11 – ***Boxplots showing the variability of recorded resistance between isolates of Pseudomonas aeruginosa received from the ICU/Surgery and the Medical ward. ICU: Intensive Care Unit; CLSI: Clinical & Laboratory Standards Institute; AK: Amikacin; CAZ: Ceftazidime; CIP: Ciprofloxacin; CT: Colistin; FEP: Cefepime; GN: Gentamicin; IPM: Imipenem; LEV: Levofloxacin; MEM: Meropenem; NET: Netilmicin; TIM: Ticarcillin–Clavulanic acid; TOB: Tobramycin; TZP: Piperacillin–Tazobactam.***

Discussion

The study provides an overview of the ecology of ESKAPE pathogens in the "Marius Nasta" Institute for Pulmonary Medicine, Bucharest, Romania.

The acronym has been publicized to draw attention to the pathogens that frequently can "escape" the effect of antibiotics.

The study has selected to characterize the implications of these pathogens in pulmonary disease.

As a tertiary center for pulmonary medicine, the "Marius Nasta" Institute deals with difficult respiratory disease from across the country. Pulmonary infectious diseases and infectious complications associated to medical or surgical care makes up an important aspect of daily clinical practice.

Enterococcus spp. are infrequently associated with respiratory pathology. This is consistent with other published studies to now, although we cannot rule out procedural and methodological confoundings [26].

S. aureus and its implication in pulmonary pathology comprises a difficult challenge in the clinical field. With a prevalence that varies widely between countries and continents, where ventilator-associated pneumonia (VAP) in the ICU represent more than three quarters of all cases, recent data suggests that current antimicrobial guidelines might be inadequate based on differences in prevalence among countries in the same region [27]. Given this fact, it has been observed that patients exhibiting other comorbidities, such as structural lung disease, other major pathologies (neoplasm, immunosuppressive states) as well as known multidrug-resistant (MDR) risk factors, such as recent hospitalization, recent prior consumption of antibiotics or systemic corticosteroids are at risk. While treatment for MRSA infections has improved with the introduction of novel fifth generation cephalosporin (*i.e.*, Ceftaroline) the treatment is very expensive and not always available. Alternatives such as glycopeptides (*i.e.*, Vancomycin) may cause kidney injury and are not always an option in severe ill patients. Because of the growing rate of MDR organisms associated with VAP, guidelines advocate the use of blood cultures and lower respiratory sampling. The issue of invasive – bronchoalveolar lavage (BAL), protected specimen brush (PSB) and biopsy – *versus* non-invasive – tracheobronchial aspirate (TBA) – seems to have been resolved through expert opinion consensus, but further study in this field is still needed [5].

Klebsiella spp. is an important *Enterobacter*iaceae, oftentimes implicated in hospital-acquired pathology, like VAP. Our results show already high levels of resistance to third generation cephalosporinases and possible "smoldering" carbapenemase reservoir [28]. Association of beta-lactams resistance with the resistance to other key antibiotic classes (quinolones, aminoglycosides) poses the clinician in grave difficulty [29]. New screening tests are needed to elucidate the presence of different resistance mechanisms [30], allowing for timely information of the clinician and timely infective control actions.

The isolates of *A. baumannii* that we investigated are extremely worrisome. For all antibiotics, except Colistin, the isolates present resistance in excess of 50%. *A. baumannii* membrane impermeability leads to difficulty in antibiotics traversing the membrane and reaching their targets [31, 32]. This leads to the fact that, even in the setting of relatively slow/weak hydrolyzing enzymes, the phenotypic impact on antibiotic resistance is high [33]. Further work needs to be done in our center, to establish the role of community infections and hospital acquired infections, the clonality and the predominant underlying resistance mechanisms. Also, as other studies showed, *A. baumannii* was the most frequently isolated pathogen from the ICU wards [34, 35].

P. aeruginosa is a well-established respiratory pathogen that "targets" sensitized patients (*i.e.*, that have underlying pathology or treatments that are risk factors for infections). As opposed to *A. baumannii*, the number of isolates is greater, with many coming from the community setting (patients with COPD, bronchiectasis) as well as hospital settings (patients with VAP) [36, 37].

Enterobacter spp. was not analyzed, due to very few samples. We conclude that *Enterobacter* and its resistance profile play a small role in our patients.

Romanian speaking readers may also be interested in reading about the resistance of *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Escherichia coli* in the center in 2015, as previously reported [38].

New strategies are needed in order to combat these pathogens. Repurposing of old antimicrobials has taken center stage for Gram-negative bacteria, with new (respiratory, intravenous) delivery systems for Colistin [39, 40] and novel pharmaceutical forms (injectable) of Fosfomycin [39, 41] being proposed. New inhibitors promise to better fight resistance mechanisms [12, 42, 43]. Modification of old antibiotics and development of new ones is a time consuming process, but recent endeavors have produced remarkable progress [44, 45]. Yet even new antibiotics rapidly suffer from antibiotic resistance [46].

The limitations of the study arise from the dataset available. Our data comes from a single center, which precludes generalizability. We relied on the laboratory data, little clinical information could be integrated in the final analysis, so a number of different clinical entities are overlapping. That being said, EKAPE pathogens are more rarely implicated in CAP, with another publication from Romania [47] implicating them in 23% (n=76) of 330 radiology-confirmed CAP, in which an etiological agent could be identified. In yet another retrospective study of 15 years, out of 4549 cases of CAP evaluated, 1597 (35%) presented an etiological diagnosis and *P. aeruginosa*, *Enterobacter*iaceae and *S. aureus* made up 6% (n=94) [48]. Of note, they reviewed the microbiology of all patients with new radiological infiltrates, regardless of underlying respiratory pathology (like COPD, chronic kidney failure, etc.).

Most of the literature on the ESKAPE pathogens focuses on the prevalence in high-vulnerability individuals: cancer patients undergoing chemotherapy [8] and transplant patients [49, 50]. However, data on overall respiratory infections are missing. Moreover, certain conditions predispose patients to iterative antibiotic treatments, thus favoring the appearance of drug resistance [51].

The strengths of our study are the ward-centric approach to resistance analysis. This describes the ecology of some of the most problematic pathogens of both the ICU/Surgery and Medical wards. This can help the clinician taper antibiotic usage based on the local susceptibility patterns and according to patient's risk factors, as presented in specialty literature [48].

Conclusions

Studies of the epidemiology of LRTI in Romania are sparse. The need to better understand the spectrum of bacterial resistance is paramount. Further work will need

to focus on generating and disseminating therapy protocols and an active surveillance system. As to the moment when submitting this article, there is no systematic national evaluation of the landscape of bacterial resistance. This work adds to the growing body of literature that needs to raise questions as to the levels of resistance that doctors are facing in their routine clinical practice, the correlation with antibiotic consumption outside hospitals and the overall impact that this has on morbidity, mortality and costs of providing medical care.

Conflict of interests

The authors declare that they have no conflict of interests.

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Received: September 24, 2016

Accepted: December 14, 2017