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ORIGINAL RESEARCH

Distribution of NDM1 Carbapenemase-Producing *Proteeae* Strains on High-Risk Hospital Wards

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Background: Carbapenem-resistant *Proteeae* (CRP) is a group of multidrug-resistant (MDR) microorganisms that raise special treatment problems due to their intrinsic resistance to colistin. In this study, our aim is to provide a phenotypic and molecular characterization of the carbapenemases secreted by CRP strains isolated from inpatients from an intensive care unit (ICU) and surgical wards, as well as the identification of the risk factors involved in their acquisition.

Methods: An observational, cross-sectional study was performed which included all *Proteeae* strains isolated in samples from inpatients on high-risk wards of the largest university hospital in Western Romania, from July 2017 to April 2019. Meropenem-resistant strains (N=65) with MIC ≥16 µg/mL were subjected to a singleplex PCR assay for the detection of *bla*NDM, *bla*VIM and *bla*CTX-M genes. The analysis of risk factors was performed by logistic regression.

Results: Out of 8317 samples that were processed, 400 *Proteeae* strains were isolated: 64% belonging to the genus *Proteus*, 26.75% to the genus *Providencia* and 9.25% to the genus *Morganella*. Most CRP strains (N=56) were of MBL type, and 55 had the *bla*NDM gene as the prevalent gene substrate. *P. stuartii* was the main species that provided the circulating MDR strains. Most CRP strains came from patients admitted to ICU, being isolated mainly from bronchial aspirates and blood cultures. Multivariate analysis revealed 3 independent risk factors – mechanical ventilation>96h (HR: 40.51 [13.65–120.25], p <0.001), tracheostomy (HR: 2.65 [1.14–6.17], p = 0.024) and prolonged antibiotic therapy (HR: 1.01 [1.00–-1.02], p = 0.03).

Conclusion: There is a significant increase in the incidence of CR *P. stuartii* strains, the MBL-*bla*NDM type being predominant. These strains presented various other resistance mechanisms, being often extremely difficult to treat and led to an excess of lethality of 27.16%.

Keywords: carbapenem-resistant *Proteeae*, intensive care unit, *P. stuartii*

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Introduction

The *Proteeae* tribe includes the following genera: *Proteus, Morganella* and *Providencia*. These are opportunistic microorganisms belonging to the *Morganellaceae* family, order *Enterobacterales*[1,2] that represent a particular challenge because of their intrinsic resistance to tigecycline, colistin, nitrofurantoin and reduced intrinsic sensitivity to imipenem. These limitations make the antimicrobial therapy for infections caused by these microorganisms a particular challenge from the start. The most sensitive genus is *Proteus*, followed by *Morganella* and *Providencia*.[3,4]

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On this background of natural resistance, *Proteeae* have developed the phenomenon of acquired resistance and even multi-resistance, so that in 2017 the World Health Organization included extended spectrum beta-lactamase-producing (ESBL) and carbapenem-resistant enterobacteria (CRE) (including *Proteus* spp., *Providencia* spp. and *Morganella* spp.) in the first category of MDR pathogens, having Priority 1: CRITICAL.[5]

The enzymes responsible for *Proteeae* resistance to carbapenems, which is considered to be of high epidemiological and clinical significance, are KPC, OXA-48 and NDM, VIM and IMP metallo-β-lactamases (MBL).[6–13]

KPC is active on the classes of penicillins, cephalosporins, β-lactamase inhibitors and also on carbapenems and aztreonam, its activity being moderately inhibited by clavulanic acid.[3]

MBLs have a significant hydrolytic activity on carbapenems, but without any effect on aztreonam, which they cannot hydrolyze.[14] The association of MBL and ESBL secretion is common in MDRs isolated in samples from immunosuppressed patients.

OXA-48 has a specific spectrum of activity, hydrolyzing penicillins and carbapenems, but without any effect on cephalosporins and aztreonam. In addition, its activity on carbapenems is not as strong as that of KPCs and MBLs, which leads to the difficultly of detection by phenotypic tests.[3,14]

The genetic material responsible for enzyme secretion is frequently identified at the extrachromosomal level, on plasmids, which represent the transfer vector of the gene substrate for the MDR behaviour, especially in inpatients. They may have representatives of the same species as the target, or can be transferred, even between different species. This transfer is especially likely to occur on high-risk wards, being helped by the patient's suppressed immune system, as well as by the large number of invasive therapeutic procedures performed. This creates conditions suitable for the occurrence of infection outbreaks with MDR microorganisms belonging to the species known for this behavior, even with less frequently identified strains.[3,4,14]

Such a situation has been reported in several Romanian hospitals over the last decade, where there has been an alarming increase in the incidence of carbapenem-resistant *Proteeae* (CRP) in samples from patients admitted to high-risk wards.[15–17]

In this context, the present study aimed to provide a phenotypic and molecular characterisation of carbapenemases secreted by *Proteeae* strains isolated from patients admitted to high-risk wards in a university hospital in Western Romania and also to identify the associated factors involved in the acquisition of these strains.

Materials and Methods

Study Design and Sampling

This observational, cross-sectional study included all strains from the *Proteeae* group isolated from samples of ICU and surgical ward inpatients from "Pius Brinzeu" County Clinical Emergency Hospital Timișoara (SCJUPBT), from July 2017 to April 2019. This institution is a tertiary medical care unit with 1174 beds, of which 32 beds are in ICU (with mixed pathology, medical and surgical), and with 501 beds on surgical wards (urology, orthopedics, general surgery, neurosurgery, vascular surgery, plastic surgery).

From the total of 400 strains identified, 65 meropenem-resistant strains (MIC ≥16 µg/mL) were collected to assess the presence of *bla*NDM, *bla*VIM genes, and for those 43, out of the 65, which had associated cefepime resistance (MIC≥64 µg)/mL) we also assessed the presence of *bla*CTX-M genes.

Patients with an ICU admission of less than 1 hour, as well as those under 18 years of age, were excluded. Those discharged and then readmitted to the same ward but on a different date were included in the database only if a new species of *Proteeae* was isolated or a new resistance phenotype was identified, completely different from the one previously included.

Demographic and medical data for the patients included in this study were collected (diagnosis on admission, date of admission, length of hospital stay, discharge status, invasive therapeutic procedures and medical conditions that may increase host's vulnerability). Bacteriology diagnostic data and antimicrobial sensitivity were also taken into account. For those 65 strains subjected to molecular analysis, data regarding the type of carbapenemase and its gene substrate were collected.

Data collection was performed with the approval of the Ethics Commission of SCJUPBT (no. 149/6.02.2019), according to the policy covering patient data protection and patient confidentiality.

Microbiological Method

To avoid duplication and phenotypic changes induced by antibiotic selection pressure, only the first strain, isolated from various samples of the same patient, was included.

As a result, 400 strains from the *Proteeae* group having clinical significance were identified and studied, being isolated from samples such as bronchial aspirates, blood cultures, wound swabs, urine cultures, catheter tips, purulent secretions, peritoneal fluid, sputum cultures, biliary fluid, ascites fluid, skin fragments, genital specimens and auricular secretions.

The primary culture was performed according to the working protocol of the Bacteriology Laboratory of SCJUPBT. The identification and antimicrobial sensitivity testing were performed using the automatic system Vitek 2 Compact (BioMérieux, France), according to CLSI.[18] The reference strains used were *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 1705.

The identification of microbial mechanisms for carbapenem resistance with the detection of KPC, MBL and OXA-48 carbapenemases secreted by *Proteeae* strains was performed using the KPC/MBL and OXA-48 kit (Rosco Diagnostica, Taastrup, Denmark).

MDR strains have been defined as resistant to at least one antibacterial agent from three or more classes of antibiotics.[19]

The monthly percentage of multidrug resistance was calculated by comparing the number of MDR strains from the same tribe/species to the total number of strains belonging with that tribe/species, isolated over that month.

The monthly share of one species in the total number of *Proteeae* strains was calculated by comparing the number of strains of that species, isolated over that month, with the total number of *Proteeae* strains isolated over the same month.

Molecular Method

All CRPs were grown for 24h at 37°C in broth medium. Bacterial DNA was extracted with PureLink Microbiome DNA Purification kit (Invitrogen) according to the manufacturer's protocol, starting from a 5mL sample. DNA concentration was assessed with NanoDrop ND1000. All PCRs were performed with AmpliTaq Gold 360 Master Mix, in 0.2 mL thin-walled PCR tubes, with 25 µL reactions mix, on a Veriti 96-Well Thermal Cycler, using 10 ng of genomic DNA (Thermo Fisher Scientific).

The isolates were analyzed for carbapenemase identification using a singleplex PCR assay, according to the method described by Poirel et al.[20] Sets of primers for *bla*NDM and *bla*VIM genes were included. For the analysis of ESBL strains, the method described by Akhi et al for the *bla*CTX-M primer pair was used.[21]

The following reference strains were used for positive control: *Escherichia coli* R45 (NDM), R61 (VIM) and R1818 (CTX-M) purchased from the Université de Fribourg. For negative control, *Escherichia coli* ATCC 25922 was used.

Statistical Analysis

Data analysis was performed using IBM SPSS Statistics 20 (SPSS Inc., Chicago, IL). Continuous variables were characterized by mean values and the interval between quartiles (IQR) and the category type was characterized by value and percentage. The 95% confidence interval was calculated for all variables. Data distribution testing was performed using the Kolmogorov–Smirnov test. The numerical variables were compared with the *t*-test for independent samples (for those with Gaussian distribution), with the Mann–Whitney *U*-test for those that do not have a normal distribution, and with the chi-squared test (Fisher exact test) for the nominal ones. The bivariate correlation was achieved by applying the Pearson correlation. The variables with p<0,05 were investigated by logistic regression, choosing the model according to the Nagelkerke R^2 coefficient and the test for assessing the deviation from the theoretical model of Hosmer and Lemeshow. All statistical tests were calculated with two extremities and the threshold of statistical significance for p was considered 0.05.

Results

From July 2017 to April 2019, 8317 samples collected from ICU and surgical wards were processed. Their distribution is presented in Table 1.

From these samples, 400 strains from the *Proteeae* tribe were identified and studied. Two hundred and fifty-six (64.00%) of these strains belonged to the genus *Proteus (P. mirabilis, P. vulgaris, P. penneri)*, 107 (26.75%) to the genus *Providencia (P. stuartii, P. rettgeri)*, the least represented being the genus *Morganella (M. morganii, M.sibonii*), with 37 (9.25%) isolated strains (Table 2).

Most *Proteeae* strains came from wound swabs (39.50%, 95% CI: 34.7–44.5), bronchial aspirates (20%, 95% CI: 16.3–24.3), urine cultures (15.25%, 95% CI: 11.9–19.2), blood cultures (8.5%, 95% CI: 6.0–11.8) and catheter tips (7%, 95% CI: 4. 8 to 10.1) (Figure 1).

From these samples, 49.75% (95% CI: 44.8–54.8) of the *Proteeae* strains came from patients admitted to ICU and the remaining 50.25% (95% CI: 45.2–55.2) came from those admitted to surgical wards.

Table 1 Distribution of Samples by Clinical Wards

Department	Total	Bronchial Aspirates		Sputum		Blood Cultures		Catheter Tips		Urine Cultures		Wound Swabs		Other Samples	
		N	%	N	%	N	%	N	%	N	%	N	%	N	%
ICU	3831	1513	39.49	57	1.48	1192	31.11	303	7.9	209	5.45	353	9.21	204	5.33
General surgery	781	0	0	10	1.28	21	2.68	12	1.54	33	4.23	466	59.67	239	30.6
Neurosurgery	217	14	6.45	0	0	20	9.22	10	4.61	39	17.97	69	31.79	65	29.95
Vascular surgery	925	1	0.11	0	0	5	0.54	4	0.43	12	1.3	894	96.65	9	0.97
Plastic surgery	824	3	0.36	1	0.12	11	1.33	6	0.73	12	1.46	787	95.51	4	0.49
Urology	1095	0	0	5	0.45	31	2.83	8	0.73	991	90.5	43	3.92	17	1.55
Orthopaedics	497	5	1	1	0.201	13	2.61	3	0.6	76	15.29	375	75.45	24	4.83
Polytrauma	147	5	3.4	0	0	15	10.2	19	12.93	19	12.93	82	55.78	7	4.76
Total	8317	1541	18.53	74	0.89	1308	15.73	365	4.39	1391	16.72	3069	36.9	569	6.84

Table 2 Phenotypic and Molecular Profile of Carbapenem-Resistant *Proteeae* Strains

Species	N=400	KPC	OXA-48	MBL			*bla*CTX-M	*bla*CTX-M +KPC	*bla*CTX-M +*bla*NDM	Molecularly Analyzed Strains
				*bla*NDM	*bla*VIM	*bla*NDM +*bla*VIM				
P. mirabilis [n, %]	245 (61.25)	1 (6.25)*	0(0)	12 (75.00)	1 (6.25)	0(0)	1 (6.25)	0(0)	1 (6.25)	16 (100)
Proteus vulgaris [n, %]	10 (2.50)	/	/	/	/	/	/	/	/	0(0)
Proteus penneri [n, %]	1 (0.25)	/	/	/	/	/	/	/	/	0(0)
P. rettgeri [n, %]	13 (3.25)	0(0)*	0(0)	1 (100)	0(0)	0(0)	0(0)	0(0)	0(0)	1 (100)
P. stuartii [n, %]	94 (23.50)	3 (6.25)*	2 (4.17)*	37 (77.08)	0(0)	1 (2.08)	1 (2.08)	1 (2.08)	3 (6.25)	48 (100)
Morganella morganii [n, %]	35 (8.75)	/	/	/	/	/	/	/	/	0(0)
Morganella sibonii [n, %]	2 (0.50)	/	/	/	/	/	/	/	/	0(0)
Total [n, %]	400 (100)	4 (6.15)*	2 (3.08)	50 (76.92)	1 (1.54)	1 (1.54)	2 (3.08)	1 (1.54)	4 (6.15)	65 (100)

Note: *Percentages reported to the total number of strains, of the same species, molecularly analyzed.

Monthly percentage(s) of multidrug resistance of *Proteeae* strains correlated strongly positive with *P.stuartii* monthly share series, from the total *Proteeae* (r = 0.833, p <0.001) and strongly negative, with the series of *P. mirabilis* monthly share (r = −0.713, p <0.001). The percentage of 69.39% of *Proteeae* multiresistance variability can be explained by the *P. stuartii* percentage variability in the total of *Proteeae* strains per month (r^2 = 0.6938) (Figure 2).

Among these 400 *Proteeae* strains, 65 CRP strains were identified (meropenem MIC ≥16 µg/mL), which were subjected to genotyping in order to detect the presence of the *bla*NDM, *bla*VIM genes. The incidence of CRP strains was 16.25%, 6.25% for the genus *Proteus* and 45.79% for the genus *Providencia*.

Most of the CRP strains were isolated from patients admitted to ICU – 86.15% (95% CI: 19.9–43.4), most of them (63%) in bronchial aspirates (32.31%, 95% CI: 21.2 −45.1) and blood cultures (30.7%, 95% CI: 19.9–43.4).

The comparison of the total *Proteeae* group with the subgroup of CRP strains reveals that CR strains were more frequently isolated from bronchial aspirates and blood cultures and less frequently from wound swabs, indicating their more frequent involvement in lower respiratory tract infections and sepsis and less so in surgical wound infections (Table 3).

From a phenotypical point of view, 100% of CRP strains were resistant to imipenem, meropenem, piperacillin, ceftazidime and 95.38% to piperacillin–tazobactam (95% CI: 87.1–99.0). Resistance to carbapenems was associated with resistance to aminoglycosides: gentamicin (84.62%, 95% CI: 73.5–92.4), amikacin (55.38%, 95% CI: 42.5–67.7), fluoroquinolones: ciprofloxacin (95.38%, 95% CI: 87.1–99.0), levofloxacin (86.15%, 95% CI: 75.3–93.5), and trimethoprim-sulfamethoxazole (98.46%, 95% CI: 91.7–100,0), having major negative effects on treatment options available for these patients (Table 4).

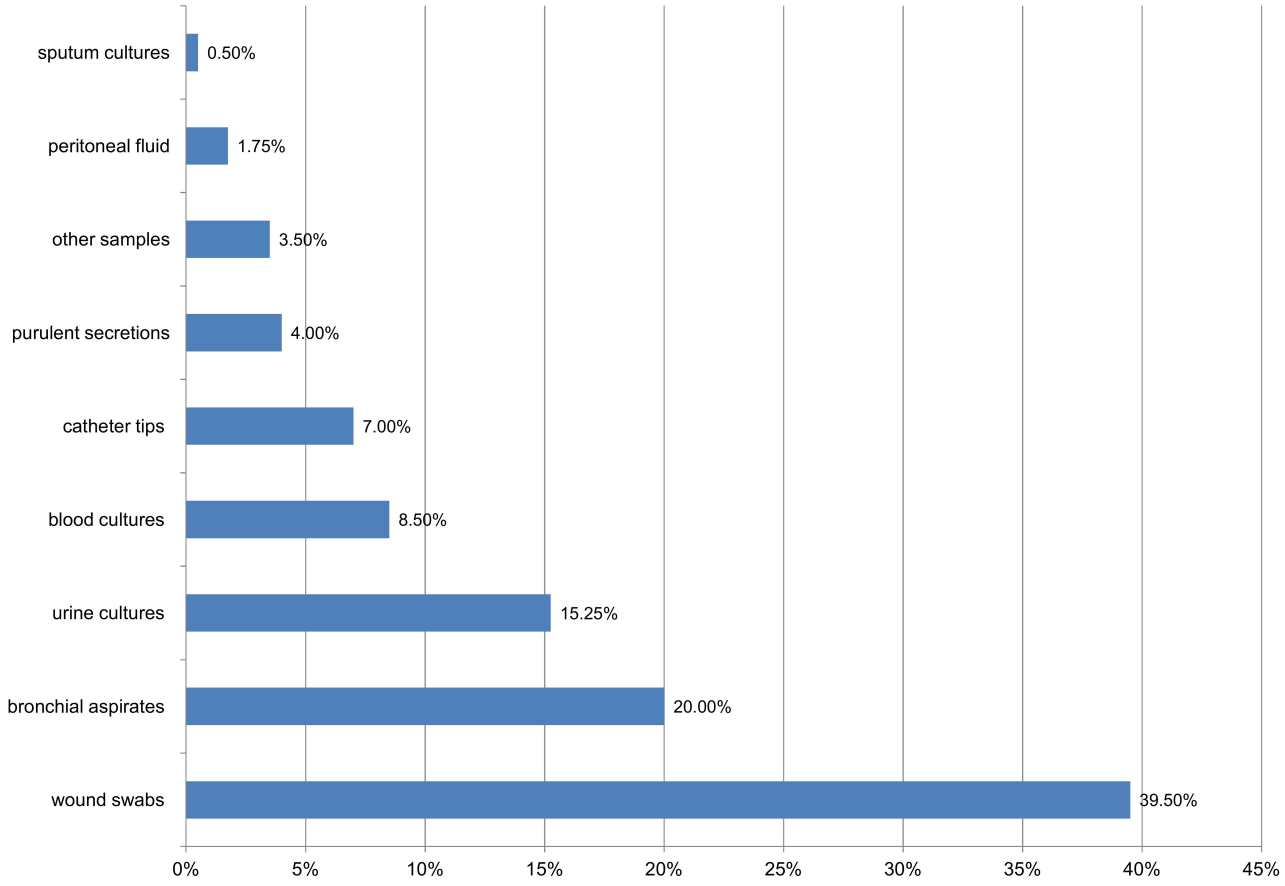

Figure 1 Distribution of *Proteeae* strains in samples.

Regarding the behaviour of fourth-generation cephalosporins, 66.15% of the strains were resistant to cefepime (95% CI: 53.4–77.4), 7 of *Proteae* strains having *bla*CTX-M gene identified by PCR test (10.76%; 95% CI: 4.4–20.9).

The identification of the bacterial resistance mechanism for carbapenems by Rosco Diagnostica test detecting KPC, MBL and OXA-48 carbapenemases secreted by *Proteeae* strains, and of gene substrate for antibiotic resistance identifying *bla*NDM and *bla*VIM genes, highlighted the CRP profile shown in Table 2.

It should be noted that the most frequently represented was MBL carbapenemase, produced by 56 of the CRP strains (86.15%; 95% CI: 75.3–93.5), having as prevalent gene substrate, in 55 strains, the *bla*NDM gene (84.62%; 95% CI: 73.5–92.4). Most of the CRP strains belonged to the *P. stuartii* species, the most prevalent being the MBL-*bla*NDM strains (61.54% of the total CRP strains), followed by the *P. mirabilis* strains of MBL-NDM type (20% of the total CRP strains). It is noteworthy that an association of *bla*NDM + *bla*VIM genes was identified in one of the *P. stuartii* strains. In two strains, one of *P. mirabilis* and the other of *P.stuartii*, although phenotypically resistant to carbapenems, no carbapenemase synthesis was identified, but they showed the *bla*CTX-M gene.

For the analysis of the predictive factors for the acquisition of CRP strains, the subgroup of CR strains was compared with the 222 *Proteeae* strains that did not meet the MDR criteria (included in the total of 400 strains that were analyzed). The results are presented in Table 5.

Moreover, the lethality of cases with CRP strains was 49.23% versus 33.43% in the total sample ($p = 0.014$) and 22.07% among those with carbapenem-sensitive *Proteeae* strains ($p < 0.001$).

Discussion

The current study aimed to analyse the behaviour of *Proteeae* strains regarding the frequency of occurrence, distribution in different samples, antimicrobial resistance profile and phenotypic/molecular characterization of CR strains, as well as identifying the risk factors involved.

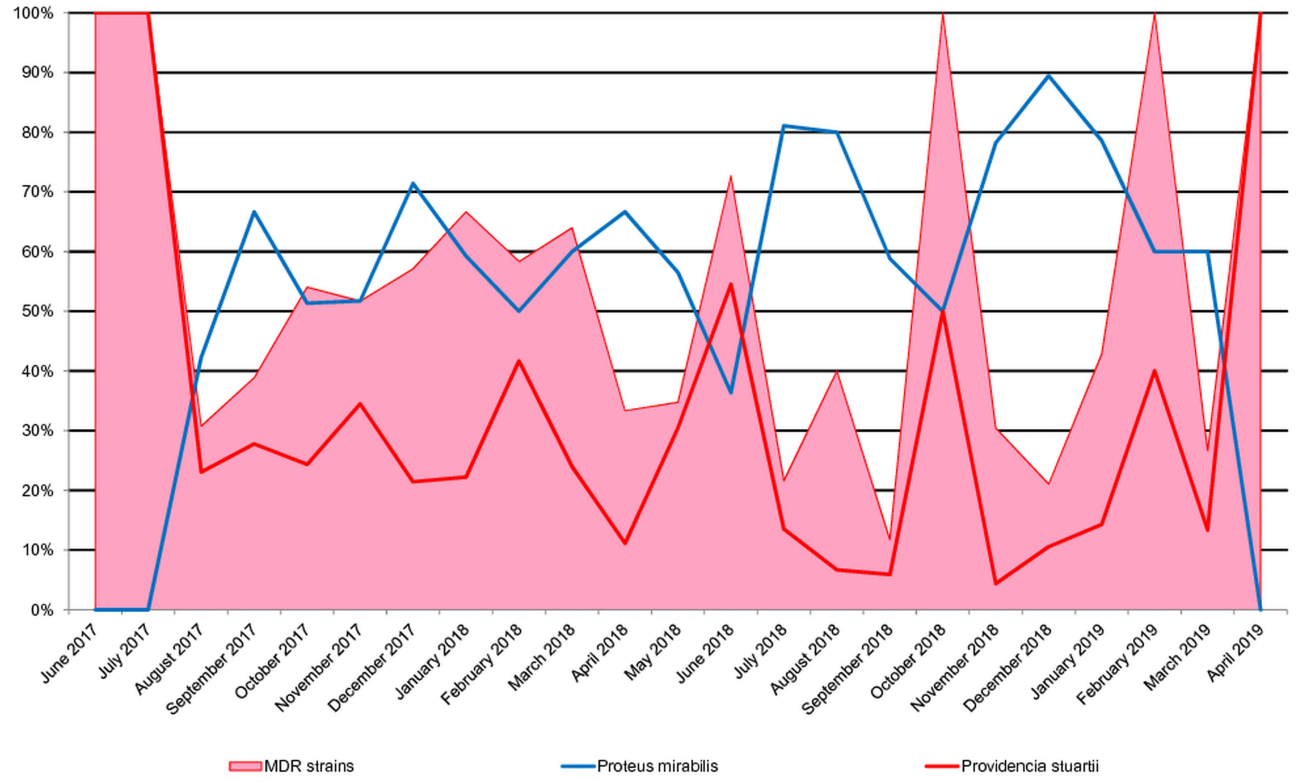

Figure 2 Evolution of the multidrug resistance of *Proteeae* strains per month in parallel with the monthly share of the two species.

The incidence of *Proteeae* strains among the total number of samples in the study was 4.81% represented mainly by *P. mirabilis* and *P. stuartii*, with a percentage of 2.94% and 1.13%, respectively and a ratio of 2.6:1. Compared with a study conducted between January 2012 and December 2013 in the ICU of the same hospital[17] a significant decrease can be observed in the ratio between these two strains in the last 5 years, due to a reduction in the percentage of *P. mirabilis* and the continuance of a relatively constant incidence of *P. stuartii* strains (the former ratio being 6:1).

We noticed a balanced distribution of the total group of *Proteeae* strains (48.25%, 50.25%) and the subgroup of *P. mirabilis* strains (44.89%, 55.11%) in the ICU and on surgical wards, while *P. stuartii* strains were identified mostly in the ICU (77.65%), where the patients are immunocompromised. *Proteeae* strains were mainly isolated in wound swabs and bronchial aspirates.

Antimicrobial testing has shown an increased frequency of MDR *Proteeae* strains (44.50%) and a correlation of multidrug resistance with the share of *P. stuartii* strains, which thus proves to be responsible for the major therapeutic difficulties in patients admitted to the ICU.

A special group of MDR strains was represented by CRP, the *P. mirabilis* – CR representing 4% of the total *Proteeae* and *P.stuartii*- CR12%, with a ratio of 3:1 between strains. What can be observed here is a reversal of the ratio between the two species for the CR subgroups and a dominance of the *P. stuartii, which* was the main species of *Proteeae* that provided the circulating MDR strains.

Regarding the phenotypical resistance to other classes of antimicrobials, most CRP strains have associated resistance to fluoroquinolones and trimethoprim-sulfamethoxazole and reduced susceptibility to aminoglycosides, especially to gentamicin. Moreover, CR *Proteeae* were

Table 3 Comparative Analysis of the Origin of *Proteeae* Strains*

Sample	Subgroup of CRP Strains		Total Group of *Proteeae* Strains		p
	n=65	%	N=400	%	
Bronchial aspirates	21	32.31	80	20.00	0.025
Blood cultures	20	30.77	34	8.50	<0.001
Wound swabs	7	10.77	158	39.50	<0.001

Note: *For the rest of the samples, there were no statistically significant differences.

Table 4 Antimicrobial Susceptibilities of *Proteeae* Strains

Antimicrobial Agent	*Proteus mirabilis* (n=16)				*Providencia stuartii* (n=48)				*Providencia rettgeri* (n=1)			
	MIC*	N	MIC*	N	MIC*	N	MIC*	N	MIC*	N	MIC*	N
Piperacillin	≥128(R)	16			≥128(R)	48			≥128(R)	1		
Piperacillin–tazobactam	≥128/4(R)	14	32/-64/4(I)	2	≥128/4(R)	47	32/-64/4(I)	1	≥128/4(R)	1		
Ceftazidime	≥64(R)	16			≥64(R)	48			≥64(R)	1		
Cefepime	≥16(R)	11	4–8(SDD)	5	≥16(R)	31	4–8(SDD)	11	≤2(S)	6	≥16(R)	1
Imipenem	≥4(R)	16			≥4(R)	48			≥4(R)	1		
Meropenem	≥4(R)	16			≥4(R)	48			≥4(R)	1		
Amikacin	≥64(R)	4			≥64(R)	31			≤16(S)	17	≥64(R)	1
Gentamicin	≥16(R)	13			≥16(R)	41	≤8(I)	4	≤4(S)	3	≥16(R)	1
Ciprofloxacin	≥1(R)	15			≥1(R)	46			≤0.25(S)	2	≥1(R)	1
Levofloxacin	≥2(R)	12	1(I)	2	≥2(R)	44	1(I)	3	≤0.5(S)	1		
Trimethoprim–sulfamethoxazole	≥4/76(R)	15			≥4/76(R)	48					≥4/76(R)	1

Note: *Breakpoints (μg/mL).

resistant to fourth-generation cephalosporins (Cefepime) in a proportion of 66.15%.

Most CRP strains (84.15%) were isolated in the samples of ICU patients, with a numerical accumulation in blood cultures and bronchial aspirates, significantly higher than in the case of the total group of *Proteeae* strains ($p < 0.001$, $p = 0.025$).

Among the carbapenemases that were identified, the best represented molecular class was that of MBLs, produced by 86.15% of CRP strains, 84.62% (n = 55) being of NDM type and only two strains of VIM type. KPC and OXA-48 carbapenemases were also identified, but less frequently. However, it was noted that all 5 KPC carbapenemase-producing strains came from neurosurgery, and the two OXA-48 strains were isolated on general surgery and neurosurgery wards.

P.stuartii– CR strains, representing the majority of the CRP group, were confirmed as producing MBL-NDM in 85.42% of cases, and less frequently KPC, OXA-48 and VIM (n=4, n=2, n=0). Only one strain showed a combination of *bla*NDM and *bla*VIM. The finding correlates with the data from the literature showing that the most common carbapenemase identified in this species was of MBL type, predominantly NDM-1[22–28] and only in isolated cases MBL-VIM-1[29–31] or MBL-VIM-19.[32] Moreover, only one study conducted in Mexico in 2015 identified a strain of *P. stuartii* secreting two carbapenemases, *bla*NDM – *bla*IMP.[33]

Regarding the *P. mirabilis* - CR strains, most of them were identified as MBL-NDM type (n=13), one MBL-VIM and one KPC type. These strains did not show a gene substrate for OXA-48 synthesis. However, studies have shown that *P. mirabilis*– CR strains are versatile in terms of carbapenemase production, MBL-NDM, VIMand IMP,[8,34–36] or KPC-producing strains being frequently reported.[6,7,9]

With regards to the *P. rettgeri* strain, this was confirmed to be MBL-NDM type. In general, these strains are rarely described in the literature and there are only a few studies conducted on larger groups. In 2005, Shiroto identified 9 strains of CR *P. retgerii*, out of a total of 495 *Proteeae* (1.81%) and 8 of these strains had the *bla*IMP-1 gene.[8] Jui-Hsuan Yang (2020) stated that 9 CR strains were identified out of a total of 142 *Proteeae* (6.33%), from 2008 to 2011.[37] In 2017, Mariappan et al identified two strains (NDM type) of *P. rettgeri* in a batch of 111 *Enterobacteriaceae*.[38]

Table 5 Analysis of Associated Factors Involved in Infections with CR *Proteeae* Strains

Variables	CRP Group n1=65	P-S Group n2=222	p	OR [95% CI]	p	HR [95% CI]
Age [median, IQR]	60 [50.00–68.00]	64.00 [54.00–72.00]	0.005	0.975 [0.96–0.99]		
Days of hospital stay [median, IQR]	45.00 [32.00–69.00]	23.00 [8.00–53.00]	<0.001	1.00 [1.00–1.01]		
Days of antibiotherapy [median, IQR]	35.00 [16.00–52.00]	9.00 [4.00–24.00]	<0.001	1.01 [1.00–1.02]	0.033	1.01 [1.00–1.02]
Number of antibiotics [median, IQR]	5.00 [4.00–7.00]	2.00 [1.00–3.00]	<0.001	1.57 [1.38–1.79]		
Days of CVC [median, IQR]	28.00 [16.00–48.00]	2.00 [0.00–12.00]	<0.001	1.03 [1.02–1.04]		
Days of urinary catheter [median, IQR]	31.00 [20.00–55.00]	0.00 [0.00–12.00]	<0.001	1.03 [1.02–1.04]		
Female [n, %]	21 (32.31)	82 (36.94)	0.493	0.81 [0.42–1.52]		
Male [n, %]	44 (67.69)	140 (63.04)				
Previous antibiotherapy Yes/No [n, %]	22 (33.85)	28 (12.61)	<0.001	3.54 [1.76–7.13]		
	43 (66.15)	194 (87.39)				
Mechanical ventilation Yes/No [n, %]	61 (93.85)	56 (25.23)	<0.001	45.21 [15.51–176.26]		
	4 (6.15)	166 (77.77)				
Length of mechanical ventilation>96 h [n, %]	59 (90.77)	47 (21.17)	<0.001	36.61 [14,42–108.45]	<0.001	40.51 [13.65–120.25]
CVC Yes/No [n, %]	61 (93.85)	129 (58.11)	<0.001	10.99 [3.85–42.83]		
	4 (6.15)	93 (41.89)				
Urinary catheter D Yes/No [n, %]	61 (93.85)	87 (39.19)	<0.001	23.66 [8.26–91.93]		
	4 (6.15)	135 (60.81)				
Hemodialysis Yes/No [n, %]	7 (10.77)	8 (3.60)	0.049	3.23 [1.00–10.32]		
	58 (89.23)	214 (96.40)				
Tracheostomy Yes/No [n, %]	41 (63.08)	28 (12.61)	<0.001	12.35 [6.18–24.91]	0.024	2.65 [1.14–6.17
	23 (35.38)	194 (87.39)				
Gastrostomy Yes/No [n, %]	2(3.08)	7 (3.15)	1.00	0.98 [0.10–5.30]		
	63 (96.92)	215 (96.85)				
Vasoactive therapy Yes/No [n, %]	46 (70.77)	43 (19.37)	<0.001	10.08 [5.14–19.92]		
	19 (29.23)	179 (80,63)				
Surgical wound Yes/No [n, %]	52 (80.00)	189 (85.14)	0.321	0.70 [0.33–1.51]		
	13 (20.00)	33 (14.86)				
Pressure sores Yes/No [n, %]	9 (13.85)	23 (10.36)	0.432	1.39 [0.56–3.38]		
	56 (86.15)	199(89.64)				
Transfusions Yes/No [n, %]	47 (72.31)	48 (21.62)	<0.001	9.47 [4.83–18.72]		
	18 (27.69)	174 (78.38)				
Nasogastric nutrition Yes/No [n, %]	57 (87.69)	53 (23.87)	<0.001	22.72 [9.68–55.26]		
	8 (12.31)	169 (76.13)				
Immunosuppressive pathology Yes/No [n, %]	5 (7.69)	12 (5.41)	0.549	1.46 [0.39–4.66]		
	60 (92.31)	210 (94.59)				

(*Continued*)

Table 5 (Continued).

Variables	CRP Group n1=65	P-S Group n2=222	p	OR [95% CI]	p	HR [95% CI]
Chemotherapy Yes/No [n, %]	0 (0.00)	6 (2.70)	0.342	0.00 [0.00–2.90]		
	65 (100)	216 (97.30)				
Radiotherapy Yes/No [n, %]	0 (0.00)	1 (0.45)	1.00	0.00 [0.00–133.20]		
	65 (100)	221 (99.55)				

Notes: P-S group, subsample with carbapenem-sensitive *Proteeae* strains; CVC, central venous catheter; Nagelkerke R^2=0.534; Hosmer and Lemeshow test=0.929; CI, confidence interval.

Association between *bla*NDM and *bla*CTX-M was noticed in the case of 4CRP strains, 75% of these being *P. stuartii*. One *P. stuartii* strain in which no carbapenemase synthesis was identified showed the *bla*CTX-M gene. Most studies have shown the presence of the *bla*CTX-M gene in other *Enterobacterales*, such as *E. coli* and *K. pneumoniae*, responsible for healthcare-associated infections (HCAI).[39–41]

Studies on NDM-producing pathogens *(Enterobacteriaceae, Acinetobacter, Pseudomonas*) show that they frequently associate determinants of antimicrobial resistance such as AmpC, ESBL cephalosporinases, other carbapenemases (OXA-48, VIM and KPC), as well as factors that lead to resistance to other classes of antimicrobials.[42–44] Therefore, most pathogens producing NDM-1 remain susceptible to only two bactericidal antibiotics (colistin and fosfomycin) and a single bacteriostatic one (tigecycline).[44,45] *Providencia* species, however, have intrinsic resistance to colistin and tigecycline, so that the limited number of therapeutic options available will represent a real challenge for the clinician, especially when *Proteeae* strains are associated with other MDR-GNB strains, such as *Acinetobacter* species, with sensitivity preserved only to Colistin.[46]

There are studies in the literature that correlate the use and the length of colistin therapy with the increase in the prevalence of colonization and infections caused by strains having intrinsic resistance to colistin, such as *Proteeae, Serratia marcescens, Pseudomonas mallei*, and *Burkholderia cepacia*.[15,46] Such increased colistin use was also noted in our hospital,[17,47,48] which may explain the increased incidence of infections with *Proteeae*.

The main risk factors identified in our study for CRP infection were mechanical ventilation, especially if lasting >96 h, the presence of the urinary catheter, nasogastric nutrition and tracheostomy. Less frequently the CRP infections were associated with other procedures such as CVC insertion, vasoactive therapy and transfusions, which are part of medical care in ICU. After applying the logistic regression, three independent risk factors remained: mechanical ventilation lasting more than 96 hours, tracheostomy and days of antibiotherapy, noting that the lethality excess was 27.16%.

In other studies performed in the same hospital, on other GNB, the risk factors for CRE infections were accounted for by the use of invasive medical devices, prolonged admission, antibiotic therapy, etc.[46,47,49]

Mariappan et al (2017) indicated as risk factors for CRE infections prolonged hospitalisation in ICU, mechanical ventilation, permanent medical devices, diabetes, use of several antibiotics, administration of carbapenems, focal infections or sepsis, as well as surgery.[38]

Clinical observations performed over the last 10 years in high-risk wards of our hospital have noted an upward trend and the persistence of infections with pathogens from the *Proteeae* tribe, frequently associated with bacterial MDR and low susceptibility to carbapenems.[15,17,48] Because this phenomenon has been repeatedly reported, in the current study we wanted to highlight the behaviour of these strains according to their molecular pattern, risk factors identified and patients prognosis, all the more so as there are not enough data in the literature on this topic.

Our study has however some limitations: it was performed in a single tertiary medical unit, in a single university centre, with a specific program of surveillance and control of HCAI, which does not allow the generalization of the results.

Conclusions

There is a significant increase in the incidence of CR *P. stuartii* strains, isolated especially from bronchial aspirates and blood cultures of patients admitted to ICU and surgical departments, the MBL-*bla*NDM type being predominant.

These strains presented various other resistance mechanisms, being often extremely difficult to treat and led to an excess of lethality of 27.16%. Mechanical ventilation lasting for more than 96 hours, tracheostomy and prolonged antibiotic therapy were the three independent risk factors identified.

In view of this, a continuous optimization of the antibiotic stewardship policy should be considered, especially with regards to the use of colistin (but not only), in order to reduce the selective pressure exerted on bacteria with natural resistance to this antibiotic. Such an approach will also have a positive effect on reducing the mortality rate, the length of hospital stay and implicitly the costs.

Abbreviations

CLSI, Clinical Laboratory Standards Institute; *bla*, β-lactamase genes; CR, carbapenem resistant; CRE, carbapenem-resistant enterobacteria; CRP, carbapenem-resistant *Proteeae*; CTX-M, cefotaximase-Munich; ESBL, extended spectrum beta-lactamase; GNB, Gram-negative bacilli; HCAI, healthcare associated infections; ICU, intensive care unit; IMP, imipenemase; KPC, *Klebsiella pneumoniae* carbapenemase; MBL, metallo-β-lactamases; MDR, multidrug resistant; MIC, minimum inhibitory concentration; NDM, New Delhi metallo-beta-lactamase; OXA, oxacillinase; PCR, polymerase chain reaction; SCJUPBT, Spitalul Clinic Judetean de Urgenta "Pius Brinzeu" Timisoara ("Pius Brinzeu" County Clinical Emergency Hospital Timişoara); TEM, Temoniera; VIM, Verona integron-encoded metallo-β-lactamase; CVC, central venous catheter.

Disclosure

The authors report no conflicts of interest in this work.

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