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Research article

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Difficult to Treat *Proteeae* strains in high risk Romanian hospital departments

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Abstract

***Introduction**: Resistance to first-line antibiotics of the Proteeae strains within the difficult-to-treat (DTR) phenotype is a cause of limitation of therapeutic options. The study aimed to characterize these strains, to identify the factors that influence their acquisition and the predictive factors for the patient's evolution.* ***Material and methods**: Between July 2017 and January 2019, 400 of Proteeae strains were isolated from samples of patients admitted to intensive care units (ICUs) and surgical wards of a university hospital in Romania. The identification and testing of antibiotic sensitivity was performed using the Vitek 2 Compact system. The DTR phenotype was defined as the resistance (or intermediate resistance) to all categories of β-lactams, carbapenems and fluoroquinolones.* ***Results**: Out of 400 Proteeae strains, 21% were of the DTR type, most of them from the species Providencia stuartii and Proteus mirabilis, identified predominantly on the ICUs. The excess fatality in the DTR subsample compared to the non-DTR subsample was 16.37%. The multivariate analysis identified as independent risk factors: the number of antibiotics administered, the number of days of urinary catheterization, the presence of tracheostomy, nasogastric nutrition, respectively belonging to the species P. stuartii. The probabilities of survival were reduced by the presence of the central venous catheter (CVC), tracheostomy, by the increase of the number of hospitalization days respectively of the number of antibiotics administered.* ***Conclusion**: The DTR phenotype in the case of Proteeae strains has been associated especially with the species P. stuartii, with invasive exogenous factors and with an increased fatality.*

***Keywords:** fatality, Providencia stuartii, antibiotical resistance*

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Introduction

The emergence and widespreadness of antimicrobial resistance has led to the need to classify bacteria into resistance phenotypes. Thus, the following can be defined multidrug-resistant bacteria (MDR), non-susceptible to ≥1 agent in ≥3 antimicrobial categories, extensively drug-resistant (XDR), non-susceptible to ≥1 agent in all but ≤2 categories and pandrug-resistant (PDR) non-susceptible to all antimicrobial agents listed (1).

In 2018, Kadri et al. (2) introduced a new phenotype, difficult-to-treat resistance (DTR), which was defined taking into account the in vivo activity of antimicrobials, respectively the relationship between treatment efficacy and adverse reactions, with repercussions on the prognosis of the disease.

Therefore, DTR defines the phenotype of bacteria resistant to all first-line antimicrobials, represented by carbapenems (imipenem, meropenem and ertapenem/doripenem), broad-spectrum cephalosporins (those relevant to the respective pathogens) and fluoroquinolones (ciprofloxacin, levofloxacin, and moxifloxacin) leaving limited and highly toxic treatment options for these germs (2). Keeping the same principles, they defined the wild type/susceptible (WT) phenotypes of the isolates, sensitive to all tested classes of antibiotics (AB) (3) and usual drug resistance (UDR) of the isolates resistant to at least one tested AB, but sensitive to first-line AB (4), the latter showing a favorable clinical response to standardized therapies. These phenotypes were estabilished respecting their natural non-susceptibility.

In this context, the *Proteeae* tribe including *Proteus*, *Providencia* and *Morganella* genera (belonging to the *Morganellaceae* family, order *Enterobacterales*) (5) has a special significance, due to its natural resistance to classes considered reserve AB (polymyxins, nitrofurans and tygecycline) for the treatment of infections with MDR / XDR / DTR strains.

Consequently, the present study aimed to identify *Proteeae* strains from the samples of patients hospitalized in at-high risk departments of a university hospital in Romania, to classify them into the DTR phenotype and to study the behavior of this phenotype related to treatment options, risk factors and chances of survival in patients with these infections.

Material and method

Microbiological method

The present study included a number of 400 of non-duplicate *Proteeae* strains, isolated from the samples of patients admitted to intensive care units (ICU), Burnt-Plastic Surgery ward (ICU-CA), and to the other surgical wards of a university hospital, between July 2017 and January 2019. Primary cultivation was done according to the working protocol of the laboratory, and the identification and testing of antibiotic sensitivity was performed using the automatic system Vitek 2 Compact (BioMérieux, France), according to the CLSI standards. We used the reference strains *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 1705.

For the definition of MDR, XDR, and PDR, the categories of antimicrobials addressed to the *Proteeae* group were taken into account, as they are presented in the definition standards (1), respecting the natural resistances characteristic of each species. Consequently, the following classes were tested: Aminoglycosides (no resistance to Netilmycin was tested), Antipseudomonal penicillins + β-lactamase inhibitors, non-extended spectrum cephalosporins, extended-spectrum cephalosporins, Carbapenems, Fluoroquinolones, Folate pathway inhibitors (Trimethoprim-sulphamethoxazole), Monobactams (Aztreonam), Penicillins + β -lactamase inhibitors (Ampicillin-sulbactam). Due to the natural resistance of *Proteeae* species, the resistance to Polymyxins (Colistin), Tetracyclines, Glycylcy-

clines (Tygecycline) and Amoxicillin-clavulanic acid was not tested. In addition, the strains were not tested with Fosfomycin (specific for urinary tract infections) and Chloramphenicol (not used due to the significant side effects).

Data analysis

Data analysis was performed using IBM SPSS Statistics 20 (SPSS Inc., Chicago, IL). Continuous variables were characterized by the median and the interval between quartiles (IQR), and the category types by value and percentage. For all variables, we calculated a 95% confidence interval. Data distribution testing was performed with the Kolmogorov-Smirnov test. The numerical variables were compared with the t-test for independent samples (in the case of the Gaussian distribution), respectively with the Mann – Whitney U test for those that did not have a normal distribution, and the nominal ones with the chi^2 test (Fisher exact test). The identification of the independent factors influencing the acquisition of infections with DTR strains was performed by logistic regression, with the introduction of variables with $p < 0.05$ in the univariate analysis and the choice of the model according to the Nagelkerke R^2 coefficient and the test for estimating the deviation from the theoretical Hosmer and Lemeshow model. The predictors of mortality/survival during hospitalization were investigated by Cox proportional hazards regression models, with the presentation of results in the form of hazard ratios (HR) with 95% confidence intervals (95% CI) and p-values. All statistical tests were calculated with 2 extremities and the threshold of statistical significance p was considered 0.05.

Results

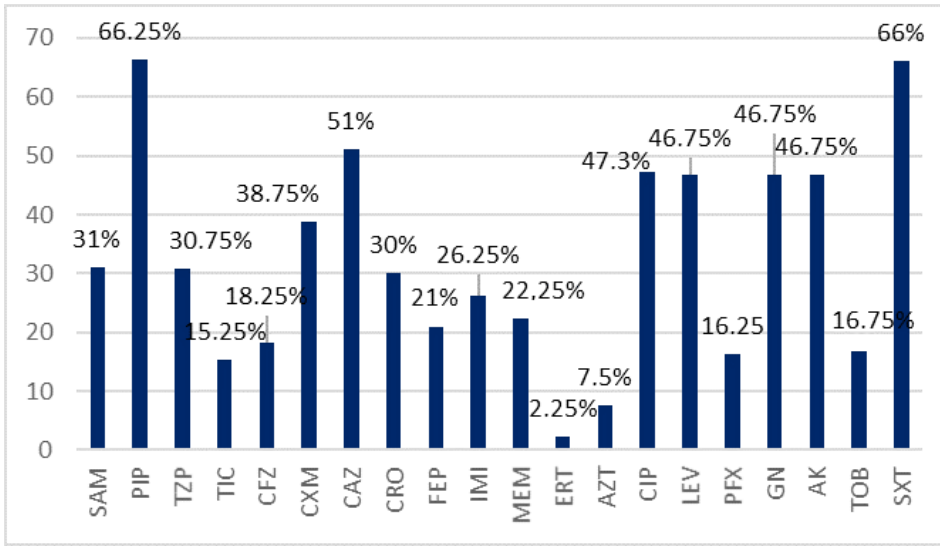
The study was conducted between July 2017 and April 2019 on a number of 400 strains of *Proteeae* identified from a total of 8317 specimens from the ICUs and surgical departments of a university hospital in Romania. The sample consisted of strains of the genus *Proteus* (64.1%), with the species *P. mirabilis*, *P. vulgaris* and *P. penneri*, strains of the genus *Providencia* (26.8%) with the species *P. rettgeri* and *P. stuartii* and strains of the genus *Morganella* (9.3%) present through the representatives of *M. morganii* and *M. sibonii*.

The working sample was numerically dominated by *P. mirabilis* and *P. stuartii* species, distributed in a different way in the clinical specimens of ICUs and surgical wards patients. In the surgical wards, the majority of specimens with *Proteeae* species were cultures from wound secretions and urine cultures, in which strains of *P. mirabilis* (72.03%, 51.11%) and *M. morganii* (11.88%, were isolated) 22.2%). In the ICU, the *P. mirabilis* strains isolated in bronchial aspirate cultures, wound secretion and blood cultures (74.03%, 63.41%, 32.14%), respectively *P. stuartii* strains identified in blood cultures, wound secretions and bronchial aspirates (67.85%, 29.26%, 23.37%) were highlighted.

The resistance of the *Proteeae* strains of the study sample is shown in figure 1.

Following the resistance to antimicrobial classes and the classification into resistance categories of the entire *Proteeae* sample, it was observed that 19% (95% CI 15.3-23.3) were of the WT type and 21% (95% CI 17.2-25.4) of the DTR (P-DTR) type, resistant to AB from the first-line of antimicrobial treatment. A percentage of 61.25 (95% CI 56.3-66.0) of the *Proteeae* was of the MDR type, 29.25% (95% CI 24.9-34.0) of the XDR type, with reduced sensitivity to antimicrobials from at most two classes of AB and 2.75% (95% CI 1.3-4.7) were PDR. 71.79% of the XDR strains were of XDR-DTR type and 28.21% of XDR showed sensitivity to at least one class of first-line AB.

The Proteeae-DTR strains (84.21%) came from the species *P. stuartii*, *P. mirabilis M. morganii*



Legend: SAM: Ampicillin / Sulbactam, PIP: Piperacillin, TZP: Pipercillin/Tazobactam TIC: Ticarcillin, CFZ: Cefixime, CAZ: Ceftazidime; CRO: Ceftriaxone, FEP: Cefepime, IMI: Imipenem; MEM: Meropenem, ERT: Ertapenem; AZT: Aztreonam, CIP: Ciprofloxacin; LEV: Levofloxacin; PFX: Pefloxacin; GN: Gentamicin; AK: Amikacin;

Fig. 1. The resistance to antimicrobial classes of the Proteeae strains

and *P. rettgeri* with an incidence and distribution on specimens/ hospitalization wards presented in Table 1. The excess fatality in the DTR versus non-DTR subsample was 16.37%.

Table 1. Descriptive analysis of nominal variables

	DTR N=84			Non DTR N=316			p
	n	%	95% CI	n	%	95% CI	
Species							
Morganella morganii	4	4.76	1.3 -11.7	31	9.81	6.9 - 13.8	0,.450
Morganella sibonii	0	0.00	0,0-0,0	2	0.63	0.1 - 2.5	1.00
Proteus mirabilis	24	28.57	19.2 - 39.5	221	69.94	64.6 - 74.9	<0.001
Proteus penneri	0	0.00	0.0 - 0.0	1	0.32	0.0 - 2.0	1.00
Proteus vulgaris	0	0.00	0.0 - 0.0	10	3.16	1.6 - 5.9	0.129
Providencia rettgeri	1	1.19	0.0 - 6.5	12	3.79	2.1 - 6.7	0.316
Providencia stuartii	55	65.37	54.3 - 75.5	39	12.34	9.0 - 16.6	<0.001
Pathological product							
Bronchial aspirate	26	30.95	21.3 - 42.0	54	17.08	13.2 - 21.8	0.004
Tegument fragment	3	3.57	0.7 - 10.1	7	2.21	1.0 - 4.7	0.444
Blood culture	17	20.23	12.3 - 30.4	17	5.38	3.3 - 8.6	<0.001
Wound secretion	10	11.90	5.9 - 20.8	148	46.83	41.2 - 52.5	<0.001
Vaginal secretion	1	1.19	0.0 - 6.5	0	0.00	0.0 - 0.0	0.210
Sputum	1	1.19	0.0 - 6.5	1	0.32	0.0 - 2.0	0.376
Urinalysis	11	13.09	6.7 - 22.2	50	15.82	12.1 - 20.4	0.536
Catheter tip	15	17.86	10.4 - 27.7	13	4.11	2.3 - 7.1	<0.001
Others	0	0.00	0.0 - 0.0	26	8.22	5.5 - 12.0	0.006

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	DTR N=84			Non DTR N=316			p
	n	%	95% CI	n	%	95% CI	
Ward							
ICU	48	57.14	45.9 - 67.9	102	32.38	27.2 - 37.8	<0.001
ICU-CA	20	23.81	15.2 - 34.3	23	7.28	4.8 - 10.9	<0.001
Surgery	0	0.00	0.0 - 0.0	49	15.51	11.8 - 20.1	<0.001
Plastic Surgery	2	2.38	0.3 - 8.3	32	10.13	7.1 - 14.1	0.023
Vascular Surgery	0	0.00	0.0 - 0.0	46	14.56	11.0 - 19.0	<0.001
Neurosurgery	5	5.95	2.0 - 13.3	4	1.26	0.4 - 3.4	0.023
Ortophaedics and Trauma	2	2.38	0.3 - 8.3	18	5.69	3.5 - 9.0	0.271
Politrauma ward	2	2.38	0.3 - 8.3	2	0.63	0.1 - 2.5	0.196
Urology	5	5.95	2.0 - 13.3	38	12.05	8.8 - 16.3	0.110
Others	0	0.00	0.0 - 0.0	2	0.63	0.1 - 2.5	1.00
Transfer ward							
/	41	48.81	37.7 - 60.0	214	67.72	62.3 - 72.8	0.001
Surgery	3	3.57	0.7 - 10.1	13	4.11	2.3 - 7.1	1.00
Plastic Surgery	0	0.00	0.0 - 0.0	6	1.89	0.8 - 4.3	0.351
Diabetes and Nutrition	1	1.19	0.0 - 6.5	5	1.58	0.6 - 3.9	1.00
Neurosurgery	16	19.05	11.3 - 29.1	37	11.71	8.5 - 15.9	0.077
Neurology	4	4.76	1.3 - 11.7	12	3.80	2.1 - 6.7	0.688
Ortophaedics and Trauma	16	19.05	11.3 - 29.1	12	3.80	2.1 - 6.7	<0.001
Mun. Hospital Caransebes	1	1.19	0.0 - 6.5	0	0.00	0.0 - 0.0	0.210
Urology	1	1.19	0.0 - 6.5	4	1.26	0.4 - 3.4	1.00
Others	1	1.19	0.0 - 6.5	13	4.11	2.3 - 7.1	0.317
Status at discharge							
Worsen	1	1.19	0.0- 6.5	4	1.26	0.4- 3.4	1.00
Improved	28	33.33	23.4- 44.5	123	38.92	33.6- 44.6	0.347
Decesed	39	46.43	35.5- 57.6	95	30.06	25.1- 35.5	0.004
Stationary	3	3.57	0.7- 10.1	18	5.69	3.5- 9.0	0.586
Cured	13	15.47	8.5- 25.0	76	24.05	19.5- 29.2	0.093
Endogenous & exogenous factors							
Gender - F	31	36.90	26.6- 48.1	114	36.07	30.8- 41.7	0.888
Gender - M	53	63.09	51.9- 73.4	202	63.92	58.4- 69.2	
Urinary catheter	76	90.47	82.1-95.8	163	51.58	45.9-57.2	<0.001
Chemotherapy	0	0.00	0.0-0.0	6	1.89	0.8-4.3	0.350
CVC	75	89.28	80.6- 95.0	206	65.18	59.7- 70.4	<0.001
Ventilation duration >96	70	83.33	73.6- 90.6	109	34.49	29.3- 40.1	<0.001
Decubitus beds	14	16.66	9.4- 26.4	37	11.71	8.5- 15.9	0.226
Gastrostomy	4	4.76	1.3 -11.7	14	4.43	2.5- 7.5	1.00
Hemodialysis	11	13.09	6.7- 22.2	22	6.96	4.5- 10.5	0.069
Nose / orogastric nutrition	70	83.33	73.6- 90.6	113	35.76	30.5- 41.3	<0.001
Immunosuppressive pathology	9	10.71	5.0- 19.4	17	5.38	3.3- 8.6	0.078
Surgical plague	71	84.52	75.0-91.5	266	84.17	79.7- 88.0	0.938
Radiotherapy	0	0.00	0.0-0.0	1	0.32	0.0-2.0	1.00
Previous ICU AB therapy	28	33.33	23.4- 44.5	56	17.72	13.8- 22.5	0.001
Mechanical ventilation	73	86.90	77.80- 93.3	124	39.24	33.9- 44.9	<0.001
Vasopressor therapy	56	66.66	55.5- 76.6	82	25.95	21.3- 31.2	<0.001
Tracheostomy	54	64.28	53.1- 74.4	66	20.89	16.6- 25.9	<0.001
Transfusions	63	75.00	64.4-83.8	104	32.91	27.8- 38.4	<0.001

Legend : CI: confidence interval ; ICU: intensive care unit ; ICU-CA: intensive care unit of Burnt-Plastic Surgery Ward ; CVC: central venous catheter ; AB: antibiotics

In order to identify the independent factors (of risk and protection) that influence the acquisition of DTR strains, 2 logistic regression models were developed (Table 2) :

1. one with the introduction of endogenous and exogenous covariates with p <0.05 in univariate analysis but without covariates targeting the bacterial strain (species and samples of origin). The independent risk factors identified were: the number of AB administered, the number of days of urinary catheterization, the presence of tracheostomy and nasogastric nutrition.
2. the second with the introduction of bacterial covariates (with p <0.05) which explains 59.4% of the variation of the DTR characteristic of the strain, and in which the infection with *P. stuartii* appears as the only independent risk factor, while the origin of the strain from the wound secretion has the quality of protection factor against DTR strain.

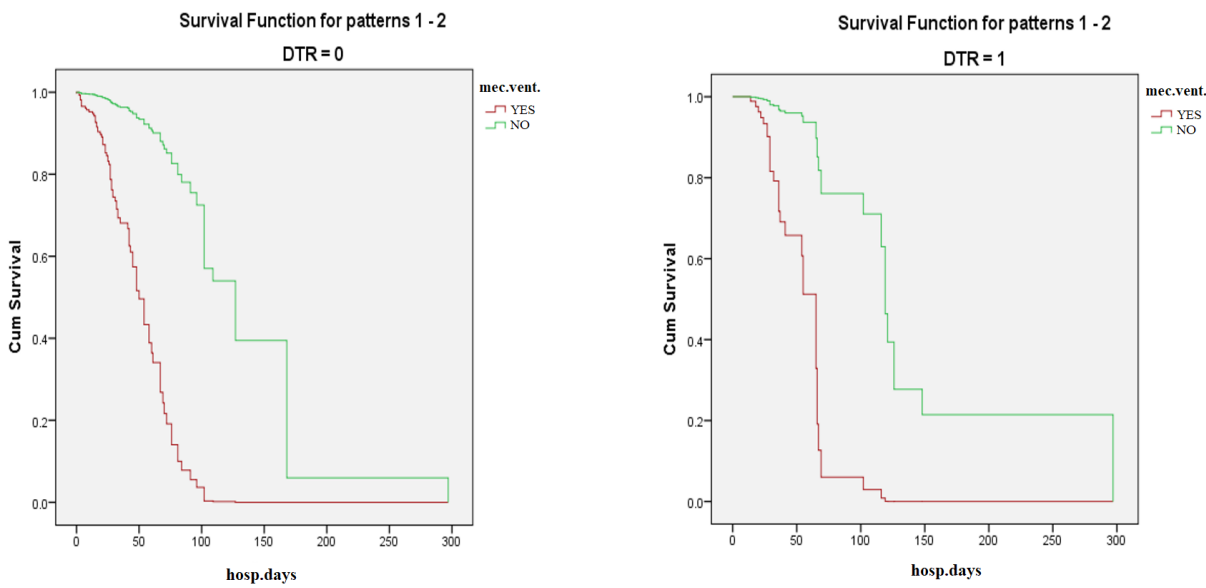
Regarding the predictors for mortality during hospitalization, the application of Cox multivariate regression identified the following variables:

- age (p = 0.001, HR = 1.024, 95% CI 1.01-1.04): the increase by 1 unit led to an increase in the probability of death by 2.3%,
- mechanical ventilation (p <0.001, HR = 10.28, 95% CI 4.03-26.27): its presence increased the probability of death by 928.8%,
- urinary catheter (p = 0.008, HR = 4.65, 95% CI 1.49-14.47): its presence increased the probability of death by 165.5%.

Analysis of survival curves confirmed the higher risks of mortality in the presence of mechanical ventilation (Figure 2):

Survival was influenced by the following factors (Figure 3):

- the presence of central venous catheter (CVC) (p = 0.024, HR = 0.68, 95% CI 0.49-0.95) which decreased the probability of survival by 32.1%,



Legend: Cum Survival: cumulative survival rate; hosp.days: hospitalization days; mec.vent.: mechanical ventilation, DTR: difficult to treat

Fig. 2. Survival curves in the subsample infected with nonDRT strains (A) and in the one infected with DRT strains (B), in those mechanically ventilated (in red) versus those without mechanical ventilation (in green)

Table 2. Factors influencing the acquisition of infections with DTR strains

Variable	DTR N1=84 Sample	Non DTR N2=316 Sample	Univariate analysis		Multivariate analysis	
			p	OR [95% CI]	p	HR [95% CI]
Age [mediane, IQR]	56.00 [44.50-77.00]	63.00 [51.00-71.00]	<0.001	0.97 [0.96-0.98]		
Number of hospitalization days [median, IQR]	54.50 [29.00-77.00]	27.00 [10.50-61.00]	<0.001	1.00 [1.00-1.01]		
Number of days of antibiotic therapy [median, IQR]	36.50 [16.00-59.00]	14.00 [5.00-32.00]	<0.001	1.01 [1.00-1.02]		
Number of antibiotics [median, IQR]	5.50 [4.00-7.00]	2.00 [1.00-5.00]	<0.001	1.43 [1.29-1.59]	0.032	1.24 [1.02-1.52] (1)
Number of CVC days [median, IQR]	29.00 [15.00-46.50]	4.00 [0.00-22.00]	<0.001	1.02 [1.01-1.03]		
Number of days urinary catheter [median, IQR]	33.00 [18.00-61.00]	2.00 [0.00-26.50]	<0.001	1.02 [1.01-1.03]	0.026	1.02 [1.00-1.04] (1)
Gender F [n, %]	31 (36.91)	114 (36.07)	0.888	1.04 [0.61-1.76]		
Gen M [n, %]	53 (63.09)	202 (63.92)				
Previous AB therapy Yes [n, %]	28 (33.33)	56 (17.72)	0.001	2.32[1,31-4,11]		
Mechanical ventilation Yes [n, %]	73(86.90)	124 (39.24)	<0.001	6.12 [3,43-10,98]		
Duration of mechanical ventilation >96 h [n,%]	70(83.33)	109(34.49)	<0.001	9.50 [4,94-18,54]		
CVC [Yes, %]	75(89.29)	206(65.19)	<0.001	4.45 [2,06-9,92]		
Urinary catheter Yes [n, %]	76(90.48)	163(51.58)	<0.001	8.92 [4,00-20,68]		
Hemodialysis Yes [n, %]	11(13.09)	22(6.96)	0.069	2.01 [0,87-4,59]		
Tracheostomy Yes [n,%]	54(64.28)	66(20.89)	<0.001	6.82 [3,92-11,90]	0.003	2.92 [1.45-5.91] (1)
Gastrostomy Yes [n, %]	4(4.76)	14(4.43)	1.00	1.08 [0,25-3,56]		
Vasopressor therapy Yes [n, %]	56(66.66)	82(25.95)	<0.001	5.71 [3,30-9,92]		
Surgical plague Yes [n, %]	71(84.52)	266(84.17)	0.938	1.03 [0,51-2,11]		
Decubitus ulcers Yes [n, %]	14(16.66)	37(11.71)	0.226	1.51 [0,73-3,08]		
Transfusions Da [n, %]	63(75.00)	104(32.91)	<0.001	6.12 [3,43-10,98]		
Nasal-gastric nutrition Yes [n, %]	70(83.33)	113(35.76)	<0.001	8.98 [4,67-17,53]	0.048	3.07 [1.01-9.30] (1)
Immuno-suppressive pathology Yes [n, %]	9(10.71)	17(5.38)	0.078	2.11 [0,83-5,26]		
Chemotherapy Yes [n, %]	0(0.00)	6(1.89)	0.350	0.00 [0,00-3,20]		
Radiotherapy Yes [n, %]	0(0.00)	1(0.32)	1.00	0.00 [0,00-146.71]		

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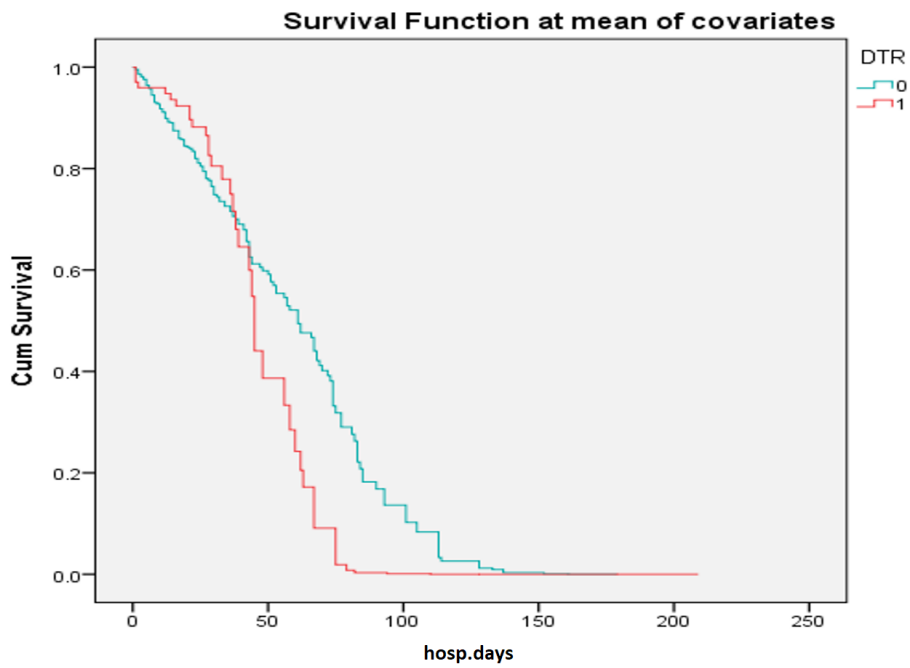
Variable	DTR N1=84 Sample	Non DTR N2=316 Sample	Univariate analysis		Multivariate analysis	
			p	OR [95% CI]	p	HR [95% CI]
Infection with *Proteus mirabilis* [n, %]	24(28.57)	221(69.94)	<0.001	0.17 [0,10-0,30]		
Infection with *P. stuartii* [n, %]	55(65.47)	39(12.34)	<0.001	13.47 [7,42-24.60]	**0.037**	**4.36 [1.09-17.38]** (2)
Bronchial aspirate [n,%]	26(30.95)	54(17.09)	0.004	2.17 [1,21-3,89]		
Blood culture [n, %]	17(20.24)	17(5.38)	<0.001	4.46 [2,05-9,75]		
Catheter tip [n, %]	15(17.86)	13(4.11)	<0.001	5.07 [2,16-11,93]		
Wound secretion [n, %]	10(11.90)	148(46.84)	<0.001	0.15 [0.07-0.32]	**0.044**	**0.17 [0.31-0.95]** (2)
ICU wards [n, %]	68(80.95)	125(39.56)	<0.001	6.49 [3.48-12.25]		
Surgical ward s [n,%]	16(19.05)	191(60.44)	<0.001	0.15 [0.08-0.29]		
Transfer ward Neurosurgery [n, %]	16(19.05)	37(11.71)	0.077	1.77 [0.89-3.52]		
Transfer section Poly- traumatology [n,%]	16(19.05)	12(3.79)	<0.001	5.96 [2.53-14.16]		

(1) or (2) - the logistic regression model



Legend: Cum Survival: cumulative survival rate; hosp.hays: hospitalization days; DTR: difficult to treat

Fig. 3. Survival curves in the subsample infected with DRT strains (in red) versus the one infected with nonDRT strains (in green).

- the presence of tracheostomy (p = 0.001, HR = 0.44, 95% CI 0.27-0.72) - decreased the probability of survival by 56.5%,
- increasing the number of days of AB therapy (p <0.001, HR = 0.97, 95% CI 0.96-0.98) by 1 unit decreased the probability of survival by 3.2%,
- the increase in the number of AB (p = 0.012, HR = 0.87, 95% CI 0.78-0.97) by 1 unit decreased the probability of survival by 12.8%,
- and the increase of the number of days of CVC (p <0.001, HR = 0.96, 95% CI 0.95-0.98) by 1 unit decreased the probability of survival by 3.6%.

Discussions

The present study aimed to identify *Proteeae* strains with DTR phenotype from clinical specimens of patients admitted to at-risk departments of a university hospital in Romania and the characterization and identification of factors influencing the acquisition of these strains, respectively of the predicting factors for patient progress. Moreover, the aim was to highlight the importance of establishing this phenotype for treatment options and patient prognosis.

Numerically, *P. mirabilis* (61.25%) was the species with the highest number of isolated strains, followed by *P. stuartii* (23.50%), both having a higher incidence than that found in other studies (6,7).

P. stuartii strains showed higher frequencies of some studied resistance phenotypes, with extremely significant differences for DTR (58.51% / 9.79%, p <0.001), XDR (74.46% / 14.28 %, p <0.001), MDR (92.55% / 51.02%, p <0.001), conversely with similar percentages for PDR strains (5.31 / 1.63%, p = 0.122), compared with the species *P. mirabilis*.

P. stuartii is a species recognized for its significant negative impact on morbidity, mortality, and treatment options (8,9). The importance of identifying this species in patient samples is also underlined by the logistic regression analysis performed. In the initial model, in which the variables related to endo- and exogenous factors were introduced, we identified as independent risk factors: the number of antibiotics administered, the number of days of urinary catheterization, the presence of tracheostomy and nasogastric nutrition. Yet, the introduction of covariates related to the strain in model 2 imposed as the only independent risk factor the *P. stuartii* infection.

In their studies, Tumbarello et al. show that independent risk factors identified for infection with extended-spectrum β-Lactamases (ESBL) producing *P. stuartii*: advanced age, previous hospitalizations, neoplastic diseases and previous antibiotic therapy (10), while the acquisition of a *P. mirabilis* MDR strain was independently associated with admission from a long-term care center, prior therapy with fluoroquinolones or oxymino-cephalosporins, urinary catheterization and previous hospitalization (11). In both studies, all the introduced and identified factors represented endogenous and exogenous variables, lacking bacterial type variables.

Survival curves confirm the high risk of mortality associated with DTR infections, especially in the presence of mechanical ventilation, a risk also identified by Tumbarello in his study on ESBL-type *P. stuartii* strains (10).

Identification of DTR-type bacteria means the loss of treatment option with high-efficiency and low-toxicity antimicrobial agents, called first-line, leading to the use of second-line agents, including aminoglycosides, tetracyclines, colistin/polymyxin B (2). In the case of Proteeae, this second-line treatment is endangered by its natural resistance to polymyxins (colistin), tigecycline, tetracycline, nitrofurantoin (1,8), with the mention that Version 3.2 EUCAST 2020 (12) states that *P. stuartii* is considered to be sensitive to Tygecycline.

Studies on MDR bacteria show that alternative treatment options are represented by the combinations of aztreonam-tavibactam, ceftazidime-avibactam and cetfolozan-avibactam (13,14). Yet, there is also research on the association of colistin with tigecycline, respectively rifampicin and linezolid in the treatment of gram-negative bacilli (15,16).

The presence of DTR strains and the treatment difficulties are indirectly reported in the literature in studies on MDR *P. stuartii* strains. Thus, it is shown that these isolates can be secretory of inducible AmpC β-lactamases, giving them resistance to monobactams and beta-lactam inhibitors (17) and it can acquire resistance to carbapenems, fluoroquinolones and aminoglycosides, through plasmid-mediated resistance mechanisms (18,19). Thus, Mahrouki (20) shows that he identified in Tunisia a strain of *P. stuartii* MDR, carrying the genes VEB-1-a / OXA-2-like and qnrA6 / aac (6) -Ib-cr (fluoroquinolone resistance) and Liakopoulos described a *P. stuartii* strain carrying genes of acquired cephalosporin (blaSHV-5 and blaVEB-1), carbapenems (blaVIM-1) and aminoglycosides resistance (rmtB) in an outbreak in Greece (21) .

On the other hand, the problem of plasmid dissemination of the DTR phenotype can be raised, Molnar et al. (22) showing that they identified in Romania *P. stuartii* strains that house the blaNDM-1 genes on IncA / C plasmids, which can be a source of spread of the phenotype, given that this conjugative plasmid has a wide range of hosts and may play a role in the spread of genes encoding carbapenemases, within the same species or between different species.

The resistance to the first-line antibiotic classes, assimilable to the DTR phenotype, has been reported in the literature for most BGNs and has been explained by the co-existence, on a bacterial level, of the responsible genetic determinants (9). Thus Poirel (23) shows that he identified 4 isolates of enterobacteria (*E. coli*, *K. pneumoniae*, *P. mirabilis*) producing NDM-1, which associated genes encoding narrow-spectrum β-lactamases (TEM type, SHV, OXA), broad spectrum β-lactamases (CTX-M, CMY), determinants of quinolones (Qnr) and aminoglycosides (ArmA) resistance, thus describing DTR-XDR phenotypes, with resistance to carbapenems, aminoglycosides and fluoroquinolones. Foldes et al. (24) showed that only for 44.18% of the positive CRE strains in their study, was the resistance given by the secretion of carbapenemases, the rest of the strains developing this behavior through other mechanisms. There are studies showing that resistance to carbapenems is given by the combinations of genes encoding carbapenemase secretion (blaOXA-48 and blaNDM), which amplifies the phenotypic expression of resistance genetic material (25) and which could be reported in the DTR phenotype.

Our study has some limitations which does not allow the generalization of the results, because it was performed in just one tertiary medical unit and a single university centre.

Conclusions

In the context of the installation of the DTR phenotype in the case of *Proteeae* species, it can be appreciated that the members of this tribe become XDR / PDR type microbial agents, with profound implications related to the treatment and prognosis of these patients, hospitalized mainly in high risk wards. The belongingness of the strain to the species *P. stuartii* represents a risk factor for contracting an infection with the DTR strain, and the combination of invasive procedures such as mechanical ventilation and bladder catheterization significantly decreases the chances of survival of these patients.

Abbreviations

DTR: difficult-to-treat
ICU: intensive care unit

P-DTR: *Proteeae* strains with difficult-to-treat phenotype
CVC: central venous catheter
MDR: multidrug-resistant
XDR: extensively drug-resistant
PDR: pandrug-resistant
UDR: usual drug resistance
WT:wild type/susceptible
AB: antibiotics
ICU-CA: intensive care unit of Burnt-Plastic Surgery Ward

Conflict of interest

The authors declare no conflict of interest.

Author contributions

Conceived and designed the experiments: MC, RM. Performed the experiments: MC, RM, SE. Data analysis: BL, MC, VS. Wrote the paper: MC. Critically revised the paper: LM, BL, MD.

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