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The role of R-plasmids in hospital strains of *Proteus*

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Abstract

The antibiotic sensitivity of *Proteus* strains isolated between 2017 and 2021 was investigated. A total of 85 *Proteus* strains were analyzed, revealing the widespread presence of poly-resistant forms of this pathogen. The examination of the antibiotic susceptibility spectrum in these strains demonstrated that those isolated from 2017 to 2021 exhibited similarities in their resistance profiles, with an expanding range of resistance observed towards gentamycin, kanamycin, carbenicillin, and others. The study identified that hospital-derived *Proteus* strains possess both conjugative and non-conjugative plasmids, distributed across different compatibility groups. It was shown that 55% of *Proteus* strains have conjugative R-plasmids, determining stability to streptomycin, gentamycin, carbenicillin, tetracycline, chloramphenicol, refer to T, J, M, N groups of incompatibility and have molecular masses 53.0; 33.0; 38.0; 49.0 MDa, respectively. In *Proteus* strains plasmids belonging to Inc-PI group of incompatibility were found. Furthermore, non-conjugative R-plasmids were identified in *Proteus* strains, conferring stability to tetracycline and streptomycin, with molecular masses of 7.2 and 5.8 MDa, respectively. Thus, the investigation of *Proteus* strains isolated in two clinics highlighted the participation of both conjugative and non-conjugative resistance plasmids in the development of hospital-associated *Proteus* strains.

Keywords: Poly-resistant; Antibiotic; R-plasmid; Hospital-associated strain; Nosocomial infectious; Transconjugants

1. Introduction

Currently, gram-negative bacteria are increasingly taking a prominent position in the landscape of nosocomial infections, pushing staphylococcal infections into the background [1, 2]. In 6-10% of infection cases caused by gram-negative bacteria, the etiological role is attributed to *Proteus* [3, 4]. Proteus-induced infections are a frequent cause of mortality in organ transplantation, particularly kidney transplants, as well as in cardiac surgeries. *Proteus* commonly leads to superinfections in patients, particularly in burn units, urological, oncological, and surgical clinics. These infections are marked by prolonged and severe courses and can even be fatal. Notably, *Proteus* bacteria with broad-spectrum antibiotic resistance play a critical role in nosocomial infections. The heightened drug resistance observed in hospital *Proteus* strains often stems from bacterial cells carrying antibiotic resistance factors known as R-plasmids [5, 6]. In fact, *Proteus* exhibits greater antibiotic resistance (with the exception of *Pseudomonas aeruginosa*) compared to other Gram-negative bacteria.

This distinctive and crucial attribute of *Proteus* creates substantial challenges when treating infections stemming from this bacterium. The aim of this study was to assess the prevalence of R-plasmids in *Proteus* strains, classify the identified plasmids, and ascertain their contribution to the development of hospital strains.

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2. Material and methods

Material for bacteriological studies was collected using sterile swabs from wounds, burn surfaces, and other relevant areas based on the patients' profiles. The collected material was inoculated onto sugar broth, blood agar, Endo or Ploskirev's medium. Pure cultures of microorganisms were obtained from the grown colonies, and their identification was performed using conventional methods involving morphological, cultural, biochemical and other properties. These methods allowed for the assignment of microorganisms to specific species [7, 8]. The susceptibility of *Proteus* strains to antibiotics was assessed using the paper disk method (indicator disk method) and the twofold serial dilution method in a liquid nutrient medium [9, 10]. Conjugative transfer of R-plasmids was conducted following the procedure described in [11, 12]. Plasmids identified in *Proteus* hospital strains were classified using the *E. coli* strain system with test plasmids, as well as the *P. aeruginosa* PAO test strain system.

Methods [13] were employed for the detection of plasmid DNA in *Proteus* strains. The removal of resistance markers was conducted using the replica method [14]. To ascertain the incompatibility groups of the plasmids, we were generously provided with tester strains containing plasmids from *P. aeruginosa* and *E. coli*.

3. Results and discussion

The study of antibiotic sensitivity among *Proteus* strains isolated between 2017 and 2021 revealed the widespread presence of multi-resistant forms of this pathogen. In total, 85 *Proteus* strains were investigated. Out of these, 60 strains were identified as *P. vulgaris*, 20 as *P. mirabilis*, and 5 as *P. morganii*. It was found that these strains exhibited resistance to 1-4 antibiotics in the range of 3.5-9.7%, to 5 antibiotics at 11.5%, to six at 18.5%, to seven at 14.4%, and to 8-10 antibiotics at 10.9-8.2%.

The *Proteus* strains isolated in 2017-2018 from the burn center of the S. Khechinashvili University Hospital (45 strains) were found to be most resistant to Pc, Ap, and Em (100%). Regarding other antibiotics, the strains exhibited resistance as follows: Sm (88.3%), Cm (84.5%), Km (75.2%), Cb (60.1%), Gm (55.2%).

The strains isolated during these same years at Unimed Kakheti (15 strains) showed almost the same pattern of antibiotic resistance: in 100% of cases, they were resistant to Pc, Ap, Em; in 93.0% to Cm and Sm; in 73.2% to Tc; in 66.0% to Cb and Gm, respectively (Table 1).

Through a comparative analysis of the prevalence of resistance traits to medicinal agents, particularly antibiotics, we studied the spectrum of antibiotic susceptibility of *Proteus* strains obtained from these two hospitals in 2019-2022. In the burn center of the S. Khechinashvili University Hospital, 15 strains of *Proteus* were isolated, while in Unimed Kakheti №2, there were 10 strains. The spectrum of resistance for *Proteus* strains from the burn center was as follows: 100% resistance to penicillin, ampicillin, erythromycin, streptomycin, and tetracycline; 93.1% resistance to chloramphenicol; 93.1% resistance to kanamycin; 86.3% resistance to carbenicillin; 80.3% resistance to gentamicin.

All strains isolated at the burn center were found to be resistant to Pc, Ap, Em, Sm, Km, Tc, and Cm. 90% of the strains exhibited resistance to Cb, and 80.0% to Gm (Table 2).

The study of the spectrum of antibiotic susceptibility among *Proteus* strains isolated in 2017-2018 and 2019-2022 revealed that their resistance profiles are quite similar. However, the range of antibiotic resistance in strains isolated during 2019-2022 noticeably increased (particularly concerning antibiotics commonly used to treat diseases caused by Gram-negative microorganisms).

To investigate the distribution of R-plasmids in clinical strains of *Proteus*, all 85 strains underwent Ethidium Orange elimination. Resistance markers were lost in 55% of the strains, most frequently for Tc and Cb (100-86.9% respectively), less often for Gm and Km (76%), Cm (56%), and Sm (87.2%).

To elucidate the nature of the genetic control behind the high drug resistance in hospital strains of *Proteus*, their ability to transfer resistance determinants via conjugation into poly-auxotrophic strains M<4262 (PAO) and J 53 was examined. We selected 47 *Proteus* strains carrying resistance markers. Crossing between donor and recipient strains revealed that all tested *Proteus* strains contained conjugative R-plasmids. These plasmids include genes that determine resistance to streptomycin (72.7%), chloramphenicol (61%), tetracycline (80%), gentamicin (53%), carbenicillin (63%) and kanamycin (72%).

Table 1 Sensitivity to antibiotics of *Proteus* strains isolated in two clinical hospitals in 2018-2019

Location of Isolations	**Number of strains**	**Resistance of *Proteus* strains to individual antibiotics**																	
		Pc		**Ap**		**Em**		**Km**		**Cm**		**Sm**		**Tc**		**Cb**		**Gm**	
		Abs.	**%**	**Abs..**	**%**	**Abs..**	**%**	**Abs..**	**%**	**Abs.**	**%**	**Abs.**	**%**	**Abs.**	**%**	**Abs.**	**%**	**Abs.**	**%**
Burn Center, S. Khechinashvili University Hospital	45	45	100	45	100	45	100	34	75.2	38	84.5	40	88.3	40	88.3	27	60.1	25	55.2
Unimed Kakheti	15	10	100	15	100	15	100	14	93.1	14	93.1	13	86.3	11	73/2	10	66.0	9	60.0
Total	60	60	100	60	100	60	100	48	84.1	52	88.8	53	87.3	51	80.7	37	63.0	34	57.6

Note: In Table 1 and Table 2: Pc - penicillin, Sm - streptomycin, Tc - tetracycline, Cm - chloramphenicol, Em - erythromycin, Km - kanamycin, Ap - ampicillin, Cb - carbenicillin, Gm - gentamicin.

Table 2 Sensitivity to antibiotics of Proteus strains isolated in two clinical hospitals in 2021-2022

Location of Isolations	**Number of strains**	**Resistance of *Proteus* strains to individual antibiotics**																	
		Pc		**Ap**		**Em**		**Km**		**Cm**		**Sm**		**Tc**		**Cb**		**Gm**	
		Abs.	**%**	**Abs.**	**%**	**Abs.**	**%**	**Abs.**	**%**	**Abs.**	**%**	**Abs.**	**%**	**Abs.**	**%**	**Abs.**	**%**	**Abs.**	**%**
Burn Center, S.Khechinashvili University Hospital	15	15	100	15	100	15	100	14	93.1	14	93.1	15	100	15	100	13	86.3	12	80.3
Unimed Kakheti	10	10	100	10	100	10	100	10	100	10	100	10	100	10	100	9	90.0	8	80.0
Total	25	25	100	25	100	25	100	24	96.5	24	96.5	25	100	25	100	22	86.1	20	80.1

Table 3 Plasmids detected in *Proteus* strains belonging to the *E. coli* incompatibility group

Location of Isolations	Plasmid	Characteristics	Incompatibility group	Molecular weight (in MDa)	Conjugation transfer frequency
Burn Center, S. Khechinashvili University Hospital	pJ378	Km Sm Tra+	J	33/0	10^{-4}
	pMT36	Sm Tc Tra+	M	38.0	10^{-3}
	pNK20	Sm Tc Km Tra+	N	49.0	10^{-4}
	pTK31	Km Tb Tra+	T	53.0	10^{-3}
Unimed Kakheti	pJ33	Km Sm Tra+	J	33/0	10^{-3}
	pM37	Sm Tc Tra+	M	38.0	10^{-4}
	pN31	Sm Tc Km Tra+	N	49.0	10^{-3}
	pTG30	Km Tc Tra+	T	53.0	10^{-3}

Table 4 Plasmids detected in *Proteus* strains belonging to the *P. aeruginosa* incompatibility group

Location of Isolations	Plasmid	Characteristics	Incompatibility group	Molecular weight (in MDa)	Conjugation transfer frequency
Burn Center, S. Khechinashvili University Hospital	pRT23	Km Cb Tc Gm Tra+	IncPI	37.0	10-4
	pRT28	Km Cb Tc Gm Tra+	IncPI	35.0	10-4
Unimed Kakheti	pRT39	Km Cb Tc Gm Tra+	IncPI	37.0	10-3
	pRT107	Km Cb Tc Gm Tra+	IncPI	35.0	10-4

The resistance of *Proteus* strains to Pc, Ap, Em, Lm, and claforan is likely determined by chromosomal genes, as the transmission of resistance determinants to these antibiotics was not detected in the resulting transconjugants. Characterizing the conjugative plasmids isolated from *Proteus* strains of patients at the burn center, it must be noted that they carry determinants of resistance to streptomycin, kanamycin, and tetracycline, and belong to incompatibility groups T, J, M, and N (Table 3), which are the plasmid groups of E. coli. However, after crossing clinical *Proteus* strains with tester strains of *Escherichia coli*, it was determined that *Proteus* strains also harbor plasmids of the IncPI group, which belong to the incompatibility group of plasmids of *Escherichia coli*.

It is known that IncPI group plasmids have a wide range of hosts and are prevalent in bacteria of the Enterobacteriaceae family (*E. coli*; *Providencia* spp; *Klebsiella* spp; *Proteus* spp; *Salmonella* spp; *Shigella* spp; *Serratia marcencens*). They play a role in the formation of hospital strains, which can lead to nosocomial infectious outbreaks.

The conjugative plasmids found in hospital *Proteus* strains isolated from patients at Unimed Kakheti also carry plasmids belonging to the incompatibility groups T, J, M, and N. In these Proteus strains, plasmids of the IncPI incompatibility group are also widespread (Table 4).

The obtained data indicate that clinical hospitals harbor *Proteus* strains containing plasmids belonging to both the *E. coli* incompatibility group and the *P. aeruginosa* incompatibility group. When studying the plasmid composition of hospital strains, transconjugants obtained by clinical isolates crossed with strains ML 4262 and J 53, were examined by the Eckhardt method for the presence of plasmids.

In the burn center, *Proteus* strains were found to carry non-conjugative R-plasmids, conferring resistance to tetracycline with molecular masses of 7.2 MDa, as well as plasmids determining resistance to streptomycin with a molecular mass of 5.8 MDa.

In *Proteus* strains from the burn center, conjugative plasmids were identified with molecular weights of 33.0 and 38.0 MDa. These plasmids convey resistance to streptomycin, kanamycin, and tetracycline, and belong to the incompatibility groups J and M. They are designated as pJ378 and pMT36, respectively. Plasmids with molecular masses of 49.0 and 53.0 MDa belong to the incompatibility groups N and T, denoted as pNK20 and pTK31, respectively.

However, as mentioned earlier, plasmids have been detected in *Proteus* strains that belong to the IncPI incompatibility group, with molecular weights of 37.0 and 35.0 MDa, and they determine resistance to tetracycline, kanamycin, carbenicillin, and gentamicin. These were designated by us as pRT23 and pRT28, respectively (Table 4).

In *Proteus* strains from Unimed Kakheti, conjugative plasmids were identified with molecular weights of 33.0, 38.0, 49.0, and 53.0 MDa. They belong to the incompatibility groups J, M, N, and T, respectively, and were designated as pj33, pM37, pN131, and pTG30. Similarly, in *Proteus* strains, as in the case of strains from the burn center, R-plasmids with molecular weights of 37.0 and 35.0 MDa were found. They confer resistance to tetracycline, kanamycin, carbenicillin, and gentamicin, and were designated as pRT39 and pRG107, respectively. These plasmids belong to the IncPI incompatibility group.

4. Conclusion

Thus, the presented results indicate the widespread occurrence of drug-resistant R-plasmids in hospital *Proteus* strains isolated from clinical settings. It is demonstrated that *Proteus* strains isolated at different times may carry R-plasmids with the same molecular weights and determine identical phenotypic traits. An interesting aspect of the identified R-plasmids is that they belong to those incompatibility groups which, among other bacterial species, have a wide range of hosts. Therefore, these results could serve as an example of the potential use of the identified *Proteus* R-plasmids as epidemiological markers. The collective data provided suggest the importance of studying the plasmid composition of *Proteus* strains isolated in hospital infections, both for detecting infection reservoirs and understanding the ways of their spread.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest amongst the authors.

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