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The 'Never-Met' Resistance of Urinary Tract Pathogens to Novel Antibiotics

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ABSTRACT

***Background*:** Antimicrobial resistance (AMR) is rapidly escalating among urinary tract pathogens, posing a pressing challenge for human and veterinary medicine. Urinary tract infections (UTIs), a common disease in humans and domestic animals like dogs and cats, are primarily caused by Gram-negative bacteria such as *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., and *Enterobacter* spp. These pathogens are notorious for their ability to develop resistance against commonly used antimicrobials. Traditional antibiotics, particularly beta-lactams and fluoroquinolones, are losing their effectiveness due to the rising resistance levels. This alarming trend necessitates exploring novel antibacterial agents to manage and treat multidrug-resistant (MDR) pathogens. This study aims to compare the efficacy of the novel antibacterial agents cefiderocol, ceftolozane/tazobactam, eravacycline, lefamulin, and omadacycline with traditional antibiotics against MDR urinary tract pathogens from humans, dogs, and cats. Understanding the effectiveness of these new agents is crucial for developing better strategies to manage UTIs and combat AMR.

***Materials, Methods & Results*:** This study collected and analyzed urinary tract pathogens from humans, dogs, and cats between 2021 and 2023. The bacterial isolates, including *E. coli*, *Klebsiella pneumoniae*, *Proteus* spp., and *Enterobacter* spp., were identified using the Vitek system. The isolates showed resistance to at least 3 different classes of antibiotics, rendering them the MDR status. The efficacy of the novel antibiotics was tested using standard disc diffusion methods. Results showed that ceftolozane/tazobactam and omadacycline demonstrated nearly 100% effectiveness against the tested MDR strains. Cefiderocol and eravacycline exhibited intermediate efficacy, indicating their potential as front-line treatments for MDR infections. In contrast, the pleuromutilin lefamulin was the least effective, likely due to its action spectrum targeting Gram-positive pathogens rather than Gram-negative bacteria predominantly responsible for UTIs. A significant finding was the observed resistance in MDR pathogens to antibiotics not yet introduced in clinical practice, suggesting a preadaptive resistance mechanism.

***Discussion*:** The study underscores the critical need for developing and implementing novel antimicrobial agents to combat the growing threat of AMR. The high efficacy of ceftolozane/tazobactam and omadacycline against MDR urinary tract pathogens presents promising options for clinical applications, offering critical alternatives to traditional antibiotics that are becoming less effective. The intermediate success of cefiderocol and eravacycline further emphasizes their potential role as first-line treatments in managing MDR infections. However, the low efficacy of lefamulin highlights the importance of selecting appropriate antibiotics based on the specific pathogen involved. The discovery of preadaptive resistance in MDR pathogens to new antibiotics underscores the necessity for continuous surveillance, judicious antibiotic use, and integrated approaches in human and veterinary healthcare settings. These findings support the 'One Health' approach, advocating for a collaborative effort to address health threats at the human-animal-environment interface. Effective stewardship programs and global monitoring are essential to preserve the efficacy of existing and future treatments, ensuring comprehensive management of UTIs. This study provides insights into the resistance patterns among urinary tract pathogens and reinforces the need for ongoing research and development in antimicrobial therapy.

Keywords: urinary tract infections, multidrug resistance, novel antibacterials, humans, pets.

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INTRODUCTION

Urinary tract infections (UTIs) are a significant health concern affecting humans and domestic animals like dogs and cats. The causative agents, primarily Gram-negative bacteria such as *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., and *Enterobacter* spp., are known for developing resistance against commonly used antimicrobials. This escalating antimicrobial resistance (AMR) presents a formidable challenge in clinical settings, necessitating a deeper understanding and innovative approaches to managing UTIs effectively [5,11].

UTIs are traditionally treated with antibacterials, but the effectiveness of these drugs is diminishing [12,14,15]. The challenge is amplified as resistant strains spread, necessitating urgent action to develop new treatments and manage antibacterial use more effectively. An interesting study detected the simultaneous presence of multidrug-resistant (MDR) *E. coli* strains from fecal sample pairs from dogs and their owners, with some strains from dogs and humans of the same household sharing phenotypic and genotypic similarities, including resistance patterns and extended-spectrum beta-lactamases genes. These data indicate within-household sharing [6,20].

Building on the understanding that MDR Gram-negative bacteria increasingly cause UTIs in humans and pets, this study aims to test these novel antibacterials' *in vitro* susceptibility profile against MDR uropathogens isolated from humans, dogs, and cats. By comparing the susceptibility profile of these new antibacterial drugs with those traditionally used in clinical settings, this research seeks to provide valuable insights into more effective management of UTIs in the face of escalating antimicrobial resistance.

MATERIALS AND METHODS

Microorganisms and drug disks

This study included the 4 most commonly found bacterial genera isolated from UTIs of humans, dogs, and cats: *E. coli*, *Klebsiella pneumoniae*, *Proteus* spp., and *Enterobacter* spp. The isolates were obtained between 2021 and 2023 and were biochemically identified using Vitek[1] (Bio-Mérieux®). Specifically, human strains included in this study were sourced from the biobanks of the Biomedicine course at Universidade Luterana do Brasil (ULBRA, Carazinho campus, Rio Grande do Sul state, Brazil), and animal strains were obtained from the Bacteriology Laboratory of the Faculty of Veterinary at Universidade Federal do Rio Grande do Sul (UFRGS). The inclusion criteria were isolates that primarily exhibited resistance to at least 3 different classes of antibiotics. Quality control strains used were *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603.

Cefiderocol (FDC), ceftolozane/tazobactam (C/T), eravacycline (ERV), lefamulin (LMU), and omadacycline (OMC) disks were imported[2]. The U.S. Food and Drug Administration approved these drugs in the years 2018-2019, except for C/T, which was approved in 2014 and is the only commercially available in Brazil, although it is not used for UTIs. The disks containing antibiotics routinely used in human and veterinary medicine were obtained commercially[3].

Antimicrobial susceptibility testing

Disc diffusion testing followed the Clinical & Laboratory Standards Institute (CLSI) guidelines [8,9]. Briefly, for disc diffusion tests, the homogenized inoculum was applied onto 150 mm Petri dishes containing Mueller Hinton agar (BD Diagnostics), followed by the placement of antibiotic discs (Table 1). After 24 h of incubation, the inhibition halos were measured, and samples were categorized as susceptible or resistant according to CLSI guidelines. For the drugs not covered by CLSI protocols, a method based on the criteria and parameters established by the Brazilian Committee on Antimicrobial Susceptibility Testing (BrCAST) in its 2021 edition was adopted [4]. This also includes drugs from new classes, some of which are not covered by either protocol. This strategic choice ensured a thorough and contextualized interpretation of the efficacy of the specific drugs under analysis.

RESULTS

A high resistance rate to cephalosporins was observed, with most bacterial species showing 100% resistance to cephalexin and cefazolin, except for *Proteus* spp. in humans, which showed 85% resistance to cephalexin and *K. pneumoniae* in humans, which showed 90% resistance to cefazolin. Moderate resistance was noted for other cephalosporins, including ceftriaxone, cefuroxime, cefepime, and ceftazidime. Fluoroquinolones exhibited reduced efficacy, particularly against *E. coli*, which showed 100% resistance to ciprofloxacin in human isolates and 40% resistance in animal isolates. Similarly, high resistance levels were found for levofloxacin and norfloxacin. High resistance was also observed for penicillin derivatives, including ampicillin/sulbactam, piperacillin/tazobactam, and aztreonam, with comparatively lower resistance to amoxicillin/clavulanic acid.

Table 1. Percentage of resistance of uropathogens in humans, dogs, and cats to antimicrobials.

Antibiotic class	Drug	*E. coli*		*K. pneumoniae*		*Proteus* spp.		*Enterobacter* spp.	
		Humans (n = 11)	Dogs/cats (n = 10)	Humans (n = 10)	Dogs/cats (n = 10)	Humans (n = 7)	Dogs/cats (n = 12)	Humans (n = 6)	Dogs/cats (n = 10)
Cephalosporins	CFE	100	100	100	100	85	100	100	100
	CFZ	100	100	90	100	100	100	100	100
	CRO	90	50	100	100	100	50	100	60
	CRX	81	60	100	100	100	50	100	60
	CPM	81	50	90	80	71	33	100	40
	CAZ	63	80	80	80	71	58	83	60
	C/T*	0	0	0	0	0	0	0	0
	FDC*	0	0	0	10	42	0	0	0
Fluoroquinolones	CIP	100	40	100	70	100	50	100	80
	LVX	100	40	100	40	100	33	0	50
	NOR	90	50	100	80	85	33	83	60
Penicillins	SBA	81	40	90	50	42	16	100	40
	PPT	90	50	90	70	100	25	66	60
	AMC	18	30	70	60	42	25	100	40
	ATM	90	50	100	80	100	66	0	0
Aminoglycosides	TOB	72	50	90	80	100	16	50	50
	GEN	45	60	100	80	100	25	100	60
	AMI	18	10	0	10	0	16	0	0
Carbapenems	IMI	9	20	90	10	100	30	0	0
	ETP	0	0	0	10	0	16	0	0
	MER	0	0	0	0	0	0	100	0
Pleuromutilins	LMU*	54	60	100	100	71	50	83	40
Tetracyclines	OMC*	0	0	0	10	0	0	0	0
	ERV*	9	10	0	40	0	0	0	0
Phosphonate	FOS	54	20	40	10	42	25	0	0

*Newly launched antibiotics. Ampicillin + sulbactam (SBA; 20 µg), amoxicillin + clavulanic acid (AMC; 30 µg), piperacillin + tazobactam (PPT; 30 µg), cefepime (CPM; 30 µg), cefazolin (CFZ; 30 µg), ceftazidime (CAZ; 10 µg), ceftriaxone (CRO; 30 µg), cefuroxime (CRX; 30 µg), cephalexin (CFE; 30 µg), gentamicin (GEN; 10 µg), tobramycin (TOB; 10 µg), amikacin (AMI; 30 µg), ertapenem (ETP; 10 µg), imipenem (IMI; 10 µg), meropenem (MER; 10 µg), aztreonam (ATM; 30 µg), ciprofloxacin (CIP; 5 µg), norfloxacin (NOR; 10 µg), levofloxacin (LVX;10 µg), fosfomycin (FOS; 200 µg), cefiderocol (FDC; 30 µg), eravacycline (ERV; 20 µg), ceftolozane sulfate + tazobactam sodium (C/T; 40 µg), omadacycline (OMC; 30 µg), and lefamulin (LMU; 30 µg).

Zero or near-zero resistance rates among the tested pathogens were observed for the novel cephalosporin/β-lactamase inhibitor combination C/T and the siderophore cephalosporin FDC. OMC demonstrated 100% efficacy against urinary tract pathogens isolated from humans and nearly 100% efficacy against those isolated from animals, with only *K. pneumoniae* showing 10% resistance in animal isolates. ERV maintained significant efficacy despite the presence of a few resistant strains. However, particularly in *K. pneumoniae*, high resistance rates were observed against LMU.

Moderate to high resistance rates were observed against aminoglycosides, particularly for tobramycin and gentamicin, with lower resistance observed for amikacin. Carbapenems were generally effective, except for imipenem (IMI), to which high resistance rates were observed in *K. pneumoniae* and *Proteus* spp. isolated from humans. Fosfomycin demonstrated moderate efficacy among human strains, with 100% effectiveness against *Enterobacter* spp. and low resistance rates in isolates from dogs and cats.

DISCUSSION

The findings of this study underscore the critical challenge posed by AMR in urinary tract pathogens. The high resistance rates observed for traditional antibiotics such as cephalosporins, fluoroquinolones, and penicillin derivatives highlight the diminishing effectiveness of these drugs. This trend is consistent with global reports of increasing resistance among Gram-negative bacteria, particularly *E. coli*, *K. pneu-*

moniae, *Proteus* spp., and *Enterobacter* spp., which are common UTI pathogens [2,14,19].

The exceptional efficacy of C/T and FDC against MDR pathogens suggests that these novel agents could be vital in the clinical management of complicated UTIs [3,16,17]. Among the tested pathogens, near-zero resistance rates were observed for both C/T, a combination of a novel cephalosporin and a β-lactamase inhibitor, and FDC, a siderophore cephalosporin. Their robust performance against MDR organisms emphasizes their potential as front-line treatments, offering critical alternatives to traditional antibiotics that are becoming less effective.

OMC also showed remarkable efficacy, with 100% effectiveness against UTPs from humans and nearly 100% effectiveness against those from animals, with only *K. pneumoniae* showing 10% resistance. As a new tetracycline antibiotic, its broad-spectrum activity and safety profile make it a promising candidate for treating UTIs, especially in settings with high rates of MDR bacteria [21]. ERV, another tetracycline derivative, showed significant efficacy despite some resistant strains, indicating its utility in addressing MDR infections [1].

Conversely, the high resistance rates of *K. pneumoniae* observed for LMU highlight the importance of selecting appropriate antibiotics based on the specific pathogen involved. LMU mainly targets common aerobic Gram-positive microorganisms, although it is active against fastidious Gram-negative bacteria. Indeed, notable gaps in antimicrobial coverage include most Enterobacterales, non-lactose-fermenting Gram-negative pathogens, *E. faecalis*, *Clostridioides difficile*, and *Bacteroides* spp. [18]. In this study, LMU demonstrated limited effectiveness against the Gram-negative pathogens prevalent in UTIs of humans, dogs, and cats.

The varied resistance patterns observed for other antibiotics, such as aminoglycosides, carbapenems, and fosfomycin, further illustrate the complexity of managing UTIs in the context of rising AMR. The moderate to high resistance to tobramycin and gentamicin contrasts with the lower resistance to amikacin, suggesting the latter's continued utility [7]. Carbapenems remained generally effective, though resistance to IMI in certain strains indicates the need for cautious use and ongoing surveillance [10,15].

Fosfomycin's moderate efficacy, particularly its 100% effectiveness against *Enterobacter* spp. and low resistance rates in isolates from animals, underscores its potential role in UTI treatment regimens [13]. However, the overall findings underscore the necessity for continuous monitoring and judicious use of antibiotics to preserve their efficacy.

CONCLUSION

This work corroborates the concerning trend of resistance to traditional antibiotics such as beta-lactams and fluoroquinolones, highlighting the critical need for new antimicrobial strategies and ongoing susceptibility monitoring to reduce the consumption of last-resort drugs. Such resistance is observed in humans and companion animals. In contrast, novel antibiotics, especially C/T, FDC, ERV, and OMC, show promising efficacy against MDR urinary tract pathogens, providing critical options in treating infections increasingly resistant to traditional antimicrobials. Notwithstanding, 100% efficacy was only observed for C/T, indicating that the tested microorganisms are already resistant to never-met newly launched drugs.

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