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Research article

Fosfomycin in the treatment of New Delhi Metallo-β-Lactamase-5 ($bla_{\text{NDM-5}}$)-producing *Escherichia coli* infection

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Abstract

The worldwide spread of Gram-negative bacteria showing pan-drug resistance raises significant concerns. The World Health Organization (WHO) designated carbapenem-resistant *Enterobacteriaceae* (CRE) as a critical priority on the global pathogen list in 2017. This issue has captured increased attention to research in the field of antimicrobial resistance, specifically concentrating on the discovery of novel antibiotics. The primary mechanism of carbapenem resistance revolves around the production of acquired carbapenemase, including class A *Klebsiella pneumoniae* carbapenem-resistant (KPC), class B New Delhi Metallo-β-Lactamase (NDM), or class D, such as OXA-48 β-lactamases. These carbapenemases are especially prevalent in Enterobacterales. Given that these various resistance mechanisms are frequently widespread, the available therapeutic options can be severely restricted. The high susceptibility rates to fosfomycin in strains with acquired resistance to carbapenems indicate the potential effectiveness of fosfomycin against such strains. The present study aimed to determine the *in-vitro* activity of aztreonam, aztreonam-avibactam, and fosfomycin against 64 *E. coli* isolates exhibiting diverse bla_{NDM} genes. From the data obtained, it can be inferred that resistance to aztreonam is 70% and drops with the combined use of avibactam. However, this combination cannot be used in the treatment of patients with diseases triggered by *E. coli* that produce $bla_{\text{NDM-5}}$. Meanwhile, all strains tested were susceptible to fosfomycin. Therefore, a remedy for elevated minimal inhibitor concentration of aztreonam, aztreonam-avibactam among $bla_{\text{NDM-5}}$-producing *E. coli* may be fosfomycin.

Keywords: Antimicrobial Resistance, Aztreonam, Avibactam, *Escherichia coli*, NDM, Fosfomycin

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Introduction

The increasing number of carbapenem-resistant *Enterobacterales* (CRE) is a significant concern for antimicrobial resistance. To combat this issue, ceftazidime-avibactam (CZA) has been introduced as a reasonable treatment option for infections caused by CRE. Avibactam enhances the effectiveness of ceftazidime in fighting infections caused by carbapenemase-producing *Enterobacterales* of Ambler class A, such as *Klebsiella pneumoniae* carbapenem-resistant (KPC), and Ambler class D, such as OXA-48. Avibactam and other β-lactam inhibitors used in clinical practice do not inhibit metallo-β-lactamases (MBLs) like New Delhi Metallo-β-Lactamase-5 ($bla_{\text{NDM-5}}$). Aztreonam is not sensitive to hydrolysis by MBLs, but it is generally affected by other β-lactamases (Emeraud et al., 2019).

Recently, the drug compound aztreonam/avibactam (ATM-AVI) underwent clinical trials to investigate its effectiveness in healing infections caused by Gram-negative bacteria, including MBL-producing ones (Bush, 2018). Since many *Enterobacterales* carry both serine-β-lactamase and MBL, the avibactam and aztreonam combination is an alternative treatment for CRE infections (Mauri et al., 2021). ATM has the ability to selectively target and bind to the penicillin-binding protein 3 (PBP3), which is highly conserved. However, some Enterobacterales strains with a specific insertion (YRIN/K) in their PBP3 have lower susceptibilities to ATM-AVI. This resistance is further increased when the strain also carries plasmid-acquired AmpC-β-lactamase and both MBLs (Sadek et al., 2020).

Urinary tract infections (UTIs) affect around 150 million individuals worldwide every year, and the primary causative agent is *E. coli* (Öztürk and Murt, 2020). The increasing antibiotic resistance, particularly due to the acquired extended-spectrum ß-lactamase (ESBL) production, is a significant challenge. To address this concern, fosfomycin has emerged as a crucial antibiotic to treat uncomplicated UTIs empirically. Fosfomycin (FOS) is widely adopted, especially in countries like Portugal, and it shows efficacy either as monotherapy or in combination with other antimicrobials against multidrug-resistant bacteria (Oliva et al., 2022). FOS is an antibiotic that works by disrupting the bacterial cell wall synthesis, resulting in bactericidal effects. The key target of this mechanism is the cytosolic N-acetylglucosamine enolpyruvyl transferase (*Mur*A), which is responsible for the initial step of peptidoglycan biosynthesis. By inactivating *Mur*A, FOS prevents the formation of the bacterial cell wall, ultimately leading to the death of the bacterial cells. This makes FOS effective in combating various bacterial infections (Falagas et al., 2010). FOS is a broad-spectrum antibiotic that is especially useful in treating UTIs caused by antibiotic-resistant strains. It is considered a first-line treatment option in empirical therapy protocols. Thus, the current study aimed to determine the *in-vitro* activity of ATM, ATM-AVI, and FOS against 64 *E. coli* strains that harbor various bla_{NDM} genes.

Materials and methods

The study involved sixty-four clinical isolates of *E. coli* collected from various sources like urine, sputum, and blood cul-

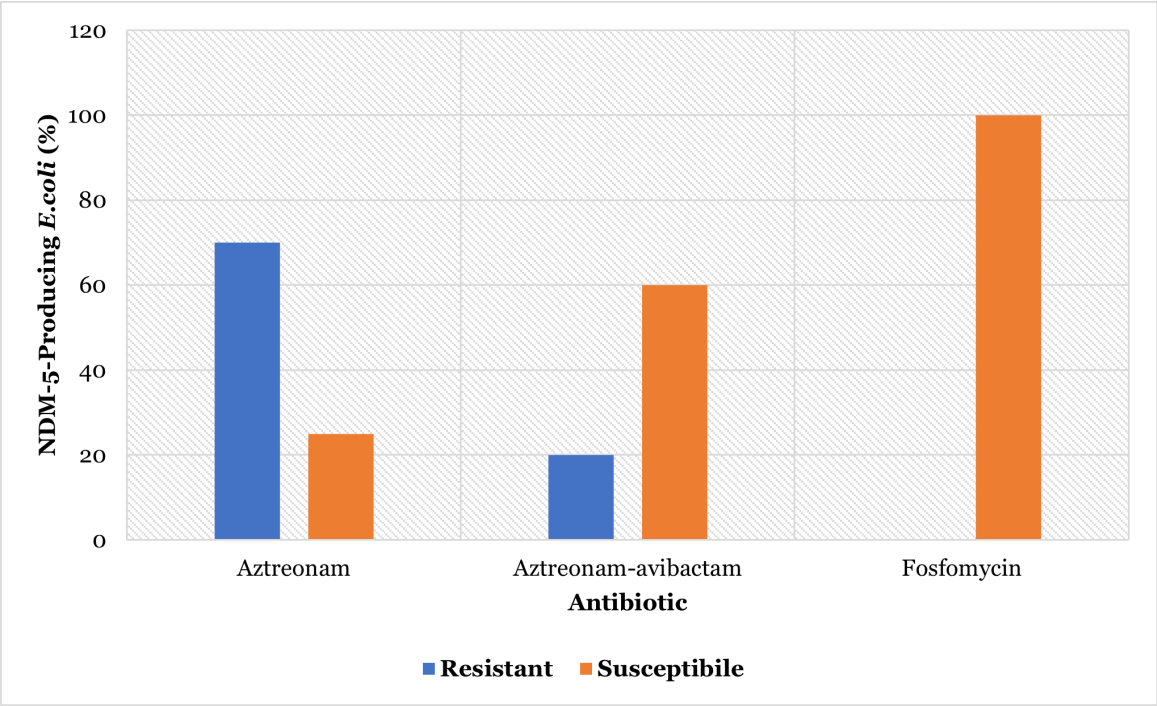

Figure 1: Comparison of the percentage of sensitive and resistant *E. coli* isolates (n= 64). The susceptibility rates for ATM, ATM-AVI, and FOS are 25%, 60%, and 100%, respectively. The resistance rates for ATM, ATM-AVI, and FOS are 70%, 20%, and 0%, respectively. ATM: aztreonam, ATM-AVI: aztreonam-avibactam, FOS: Fosfomycin.

tures in Switzerland. Aztreonam and aztreonam-avibactam were tested on these isolates to determine their Minimum Inhibitory Concentration (MIC). The strains were also screened with aztreonam (30 μg) and fosfomycin (200 μg) using the disc diffusion method to evaluate their sensitivity and resistance. The broth microdilution method in the Mueller-Hinton broth (Bio-Rad, Marnes-la-Coquette, France) was used to determine the MICs. The drugs used in the study were procured from Sigma-Aldrich (Buchs, Switzerland) and Roche (Basel, Switzerland). Avibactam was tested at a fixed concentration of 4 μg/mL. Since there is no specified breakpoint value for defining ATM-AVI resistance, the cut-off for ATM alone (>4 μg/ml; www.eucast.org/clinical_breakpoints) was arbitrarily selected. To ensure accuracy and reliability, susceptibility testing was implemented in duplicates, and the *E. coli* ATCC 25922 strain was used as a control for all testing procedures.

The most recent European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints (www.eucast.org/clinical_breakpoints) were used to interpret the results. Multiple methods were employed to evaluate carbapenemase production, including the Carba NP biochemical test (Dortet et al., 2015) and the NG-Test Carba5 assay (NG Biotech, Guipry, France) (Jenkins et al., 2020). The NG-Test Carba5 assay aims to detect the five main types of carbapenemase genes (IMP, VIM, NDM, KPC, and OXA-48).

Results and discussion

A total of 64 *E. coli* bacteria carrying NDM-5 were chosen to determine their susceptibility to ATM, using a disc diffusion assay specifically designed for ATM. The results showed that 45 of these NDM-5 *E. coli* strains were resistant to ATM, and these strains are clinically significant in Switzerland (as shown in Figure 1). 16 *E. coli* isolates were found to be susceptible to ATM, with an inhibition zone diameter of $\geq$26 mm when a 30 μg disc was used, as recommended. Three *E. coli* displayed intermediate susceptibility to ATM, with the diameter of the inhibition zone between $\geq$21 and <26mm, based on EUCAST breakpoints (www.eucast.org/clinical_breakpoints) guidelines.

Out of the 64 *E. coli* strains that were tested, 13 strains were resistant to ATM-AVI, while another 13 strains showed intermediate susceptibility to it. The isolates that showed both resistance and intermediate susceptibility to ATM were observed to have activity during the broth microdilution assay for ATM and ATM-AVI and in disc diffusion for FOS using a 200 µg fosfomycin, as shown in Table 1. All isolates were found to be resistant to ATM and ATM-AVI based on the ATM resistance breakpoint (>4 mg/L) as per the EUCAST guidelines. Notably, all isolates were susceptible to FOS. Isolates show resistance to ATM due to the presence of Ambler class A, C, and D, which degrade ATM via β-lactamases other than Ambler class B, such as NDMs. Conversely, resistance to ATM-AVI is likely linked to the presence of PBP3 variants within the collection, which reduces ATM's ability to bind with PBP3 (Sadek et al., 2020).

It has been observed that NDM-producing *K. pneumoniae* and *E. coli* have emerged in different countries, leading to outbreaks between regions, including Spain and India (Rahman et al., 2014; Pérez-Vázquez et al., 2019). This study conducted in Switzerland evaluated the use of FOS against bla_{NDM}-producing *E. coli in-vitro*. The findings indicate that the prevalence of FOS resistance in *E. coli* is low in Switzerland, with a rate of 1.4% in the community. It was also reported that the resistance rate to FOS in the USA, Asia, and Europe between 2004 and 2009 was 3.2% (Falagas et al., 2010). Although some studies after 2010 showed an increase in FOS resistance, the overall resistance rate remains below 10% (Falagas et al., 2016).

The observed *in-vitro* activity of FOS towards bla_{NDM}-producing *E. coli* in Switzerland might mirror the generally low resistance levels seen in numerous pathogens in the country (Fulchini et al., 2019). A former study carried out in Switzerland examining antimicrobial resistance in *E. coli* isolates between 2012 and 2015 reported a FOS resistance rate below 1% (Erb et al., 2018). Given the low prevalence of FOS resistance monitored in the abovementioned studies, it is tempting to point out a low risk of spread of FOS resistance among NDM-producing *E. coli*. These results suggest that FOS may still be used as a therapy for infections caused by ATM/AVI-resistant *E. coli* isolates that produce $bla_{\text{NDM-5}}$. Despite this, some FOS-resistant *E. coli*

Table 1: Susceptibility of *bla*$_{NDM\text{-}5}$-producing *E. coli* isolates. R; resistant, S; susceptible.

Strains	Aztreonam	Minimum inhibitory concentration (μg/mL)		Fosfomycin
		Aztreonam	Aztreonam-avibactam	
N6	R	>32	4	S
N8	R	>32	1	S
N21	R	>32	1	S
N57	R	32	8	S
N185	R	32	8	S
N204	R	>32	4	S
N231	R	32	4	S
N322	S	-	-	S
N415	R	32	≤0.03	S
N442	R	>32	1	S
N445	S	-	-	S
N461	R	32	1	S
N489	R	>32	4	S
N525	R	>32	2	S
N568	R	>32	0.5	S
N590	R	>32	8	S
N640	R	32	8	S
N653	R	>32	1	S
N665	R	>32	1	S
N679	S	-	-	S
N737	S	1	≤0.03	S
N775	S	32	2	S
N897	R	>32	2	S
N898	R	>32	2	S
N901	R	>32	1	S
N935	S	>32	2	S
N1013	R	>32	8	S
N1014	R	>32	0.5	S
N1071	S	-	-	S
N1076	R	32	8	S
N1081	S	1	0.5	S
N1097	S	0.125	≤0.03	S
N1115	S	-	-	S
N1146	I	16	2	S
N1153	R	>32	8	S
N1235	S	-	-	S
N1239	R	>32	2	S
N1255	R	>32	1	S
N1372	R	>32	4	S
N1416	R	>32	16	S
N1439	S	0.125	≤0.03	S
N1442	R	>32	4	S
N1452	R	>32	0.5	S
N1470	R	32	16	S
N1494	S	-	-	S
N1508	S	-	-	S
N1606	R	>32	8	S
N1612	R	>32	2	S
N1644	R	>32	2	S
N1648	R	>32	2	S
N1691	R	>32	2	S
N1707	R	>32	0.5	S
N1718	I	8	2	S
N1779	S	-	-	S
N1792	R	0.06	≤0.03	S
N1880	R	>32	1	S
N1949	R	>32	8	S
N1972	R	>32	8	S
N1980	R	>32	4	S
N1985	R	>32	4	S
N1986	R	>32	4	S
N2007	S	-	-	S
N2110	R	>32	2	S
N2132	I	16	16	S

isolates have been progressively encountered (Ríos et al., 2022). Given that $bla_{\text{NDM-5}}$-producing isolates disseminate worldwide (Rahman et al., 2014; Yang et al., 2014). FOS still provides the capacity to treat the most common community-acquired and healthcare-associated infections in humans, such as urinary tract infections (UTIs).

The ongoing and widespread consumption of FOS may lead to the development of bacterial strains that are insensitive to the antibiotic, posing a potential threat on a global scale (Oteo et al., 2009). CTX-M-15-producing urinary *E. coli* O25b-ST131-phylogroup B2 has acquired resistance to FOS (Zurfluh et al., 2020). The potential side effects of FOS may be more prevalent in patients, including symptoms such as dizziness, headaches, and vaginitis, particularly with increased FOS consumption (Hashemian et al., 2019). Additionally, it may contribute to the selection of bacteria with acquired resistance to FOS, including *Enterobacter* spp. For better treatment options, it was reported that the clinically significant synergistic interactions with FOS were primarily observed in combinations with penicillins (51%), carbapenems (43%), chloramphenicol (39%), and cephalosporins (33%) within Enterobacterales (Antonello et al., 2020). Alternatively, as of January 2020, Locus Biosciences announced the initiation of enrollment for a Phase 1b clinical study for their CRISPR-mediated phage product, LBP-EC01, devised to target *E. coli* (https://www.locus-bio.com/media/locus-biosciences-initiates-worlds-first-controlled-clinical-trial/). To provide a more up-to-date understanding of FOS resistance among MBL-producing *E. coli* in Switzerland, further studies are warranted. Ongoing surveillance and research efforts will be fundamental for staying ahead of emerging trends in antibiotic resistance and informing effective treatment strategies.

Conclusion

Our study focused on 64 $bla_{\text{NDM-5}}$-producing *E. coli* isolates, revealing a concerning prevalence of ATM resistance, particularly in clinically relevant strains circulating across Switzerland. With the AVI coupled to ATM, resistance rates in $bla_{\text{NDM-5}}$-producing *E. coli* reduced to 20% from 70%. Notably, FOS demonstrated consistent efficacy against all isolates, presenting a promising therapeutic option for infections due to these resistant strains. Despite the emergence of FOS-resistant *E. coli* isolates globally, our findings underscore the continued use of FOS, especially in tackling widespread human infections like urinary tract infections. Importantly, this work contributes novel insights as there is currently a lack of research specifically evaluating the susceptibility of collected $bla_{\text{NDM-5}}$-producing *E. coli* strains to FOS.

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