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IN VITRO SUSCEPTIBILITY PROFILES OF FTOLOZANE–TAZOBACTAM AND CEFTAZIDIME–AVIBACTAM AGAINST AMPC/ ESBL PRODUCING ENTEROBACTERIACEAE

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Abstract

Background: Antimicrobial resistance (AMR) among Gram-negative bacteria (GNB)—especially those producing AmpC β-lactamases and Extended-Spectrum Beta-Lactamases (ESBLs)—represents a major threat to global public health. These resistant organisms significantly limit available treatment options and contribute to increased morbidity and mortality. The study examined and compared the *in vitro* susceptibility profiles of carbapenem-sensitive ESBL- and AmpC-producing *Enterobacteriaceae* isolates against two newer β-lactam/β-lactamase inhibitor combinations.

Methods: Seventy-eight non-repetitive bacterial isolates were procured from clinical settings. Routine conventional microbiological methods were used for identification of all isolates. Kirby–Bauer disc diffusion (KBDD) method was used for antimicrobial susceptibility testing. Molecular detection of the blaNDM-1 gene was done through Polymerase Chain Reaction (PCR) for carbapenem-resistant isolates. The minimum inhibitory concentrations for C/A and C/T were established by E-tests. Data was analyzed using SPSS version 17.0 with P-value of <0.05 was considered statistically significant.

Results: The study included 78 patients (51 male, 27 female), with swab (43) being the most common sample type. Isolates comprised 34 *Escherichia coli (E. coli*) and 38 *Klebsiella pneumonia (K. pneumonia)*. C/A demonstrated statistically superior overall activity against *E. coli* (88.2% susceptible) compared to C/T (79.4% susceptible) (P = 0.004). Similarly, against *K. pneumoniae*, C/A showed higher susceptibility (57.9%) than C/T (50.0%) (P = 0.049). When stratified by phenotype, both C/A and C/T demonstrated 100% in vitro susceptibility against ESBL-producing *E. coli*. C/A demonstrated significantly higher susceptibility against AMPc-producing *E. coli* (50% vs. 33.3%; P = 0.008) and AMPc-producing *K. pneumoniae* (52.6% vs. 21.1%; P = 0.338). For ESBL+AMPc co-producers, C/A showed 94.4% susceptibility against *E. coli* and 85.7% against *K. pneumoniae*, generally outperforming C/T. Overall, C/A showed 52% susceptibility against AMPc-producing isolates compared to 24% for C/T (P = 0.007). Notably, C/T exhibited better activity against NDM Negative isolates (58.3% susceptible) than C/A (8.3% susceptible).

Conclusion: Compared to C/T, C/A showed superior activity against *E. coli* and *K. pneumoniae*, particularly in isolates producing AmpC β-lactamase. While both agents were highly effective against ESBL producers, C/A consistently showed higher susceptibility rates across various categories, with

a notable exception for NDM Negative isolates where C/T was more active. The findings emphasize the need for region-specific susceptibility data to optimize empirical and definitive therapy against MDR GRB.

Introduction:

Antimicrobial resistance in GNB—especially among members of the *Enterobacteriaceae* family and non-fermenting organisms—has emerged as a critical global health threat in the 21st century [1,2]. This escalating crisis is characterized by the diminishing efficacy of existing antibiotic therapies, resulting in higher rates of illness and death, along with significant financial burdens on healthcare systems worldwide [1,2]. The extensive dissemination of ESBL and AmpC β-lactamase-producing bacteria is a key driver of this escalating resistance pattern [8]. These enzyme groups are globally recognized as major contributors to resistance, with India reporting particularly high prevalence rates of ESBL and AmpC at 40% and 36.5%, respectively [8,5].

The predominant driver causing resistance to broad-spectrum β-lactam antibiotics in bacteria is ESBL and AmpC enzymes expression, which inactivate penicillins, multiple generations of cephalosporins, and aztreonam through enzymatic hydrolysis. This enzymatic breakdown prevents these antibiotics from effectively binding to their target sites, thereby allowing the bacteria to survive and proliferate despite treatment. [3,4]. While ESBLs typically lack the ability to hydrolyze cephamycins and carbapenems, overexpression of AmpC β-lactamases can result in the breakdown of cephamycins such as cefoxitin [5]. Notably, ESBLs enzyme is inhibited by β-lactamase inhibitors, however AmpCs β-lactamases shows limited susceptibility to these agents but are better addressed with diazabicyclooctane inhibitors such as avibactam. [6]. Nonetheless, many ESBLs are also capable of conferring resistance to fourth-generation cephalosporins, such as cefepime and cefpirome [7]. The widespread occurrence of these resistance mechanisms has resulted in carbapenems becoming the preferred treatment for severe infections caused by ESBL- and AmpC-producing *Enterobacteriaceae*. However, this dependence has contributed to the rising prevalence of carbapenem-resistant strains, highlighting the urgent need for effective carbapenem-sparing alternatives to preserve the efficacy of existing last-line therapies [1,2].

To address the escalating threat of multidrug-resistant (MDR) Gram-negative infections, new β-lactam/β-lactamase inhibitor combinations have been developed as promising therapeutic alternatives. Among these, C/T and C/Ahave gained particular attention for their clinical potential [9–11]. C/T consists of ceftolozane, a newer-generation cephalosporin, combined with tazobactam, a β-lactamase inhibitor primarily targeting Ambler class A enzymes [15–17]. Ceftolozane exerts its antibacterial effect by binding to penicillin-binding proteins (PBPs), thereby inhibiting bacterial cell wall synthesis, with a particularly strong affinity for PBPs 1b, 1c, 2, and 3. [15–20].

This combination has shown strong *in vitro* efficacy against both *E. coli* and *K. pneumoniae* which are ESBL-producing, with reported susceptibility rates exceeding 90% and reaching up to 98% [10]. Additionally, C/T has shown significant efficacy against *Pseudomonas aeruginosa*, including MDR strains [13,14]. Pooled analyses of Phase 3 clinical trials have demonstrated its effectiveness in treating cUTIs and intra-abdominal infections attributable to ESBL producers [2,23].

However, its activity against AmpC-producing *Enterobacteriaceae* is variable, primarily due to tazobactam's limited inhibitory effect on class C β-lactamases [1,10]. Notably, ceftolozane possesses enhanced cellular penetration, even in the presence of efflux mechanisms, and demonstrates greater stability against AmpC enzymes compared to ceftazidime [10,13,14].

C/A is a novel combination agent that represents a mechanistically distinct advancement to treat Gram-negative resistant infections. It pairs ceftazidime, a third-generation cephalosporin that exerts its antibacterial effect by preferentially binding to penicillin-binding protein 3 (PBP 3), with avibactam, a broad-spectrum, non-β-lactam β-lactamase inhibitor [15–20]. Notably, avibactam inhibits key β-lactamase classes, including Ambler class A (ESBLs and KPC), class C (AmpC), and selected class D enzymes such as OXA-48 [10–11]. This wide inhibitory spectrum allows avibactam to restore the efficacy of ceftazidime against various MDR organisms, particularly those producing

ESBLs, AmpC, and selected carbapenemases [3–4, 11–12]. A key pharmacodynamic feature of avibactam is its reversible binding to β-lactamases, which facilitates sustained inhibition through recycling of the inhibitor molecule [10]. Global surveillance efforts, including the INFORM study, have demonstrated that C/Aretains activity against over 97% of ESBL- and AmpC-producing, as well as carbapenem-resistant *Enterobacteriaceae* isolates—many of which are resistant to most conventional antibiotics [24,25]. Evidence from a meta-analysis of randomized trials has shown that C/A achieves clinical cure rates comparable to carbapenems for ESBL-producing *Enterobacteriaceae* infections, and shows promising activity against AmpC producers, although further clinical validation for the latter is ongoing [4].

Despite both C/T and C/A show favorable *in-vitro* activity profiles, the comparative susceptibility against ESBL- and AmpC-producers to these agents is clinically significant and nuanced. Distinct divergences in their β-lactamase inhibition profiles contribute to varying efficacies against different resistance mechanisms. For example, although avibactam is more active against a wider group of β-lactamases such as AmpC and certain carbapenemases, tazobactam's activity is more limited to class A enzymes. Furthermore, the potential for the emergence of resistance to these new drugs, particularly among AmpC producers through mechanisms such as structural mutations or increased enzyme expression, may significantly influence long-term therapeutic efficacy and necessitates careful monitoring [26]. Variability in susceptibility rates observed among different bacterial species and across diverse geographic regions further underscores the critical necessity of robust comparative analyses to accurately guide empirical and targeted therapy, preserve the efficacy of these valuable antimicrobial agents, and inform local and national antimicrobial stewardship strategies [1,5,25].

In light of these evolving challenges and the pressing global demand for useful carbapenem-sparing alternatives, a systematic evaluation of the comparative susceptibility profiles of C/T and C/A among carbapenem-sensitive ESBL and AmpC-producing *Enterobacteriaceae* is essential. Such studies are crucial for delineating the optimal clinical applications of each agent, facilitating informed treatment decisions, and contributing to the development of evidence-based treatment algorithms for MDR Gram-negative infections. This research particularly seeks to assess the vulnerability of carbapenem-sensitive ESBL and AmpC Enterobacteriaceae isolates to C/A and C/T.

Materials and Methods

Bacterial Strains and Ethical Considerations

We obtained N=77 unique (non-duplicate) bacterial isolates from inpatient wards and outpatient departments (OPDs) of Safdarjung Hospital. These isolates were not prospectively collected; they were recovered from routine clinical specimens and preserved as pure stocks within the Microbiology Department's repository, which contains human biological material. For the purposes of this study, the archived isolates were retrieved and properly labeled. Ethical approval was taken from the institutional ethics committee due to the use of patient-derived material and associated clinical data. Bacterial identification was performed using standard conventional microbiological methods.

Assessment of Antimicrobial Susceptibility

Antimicrobial susceptibility was carried out using the KBDD technique, following the CLSI guidelines. The antibiotics tested included 30µg ampicillin, 20µg/10µg amoxicillin–clavulanic acid, 10µg gentamicin, 30µg amikacin, 30µg cefotaxime, 1.25/23.75µg trimethoprim–sulfamethoxazole, 30µg ceftazidime, 5µg ciprofloxacin, 100µg piperacillin, 100µg/10µg piperacillin–tazobactam, 30µg ceftriaxone, 30µg cefoxitin and10µg imipenem. Inhibition zone diameters were interpreted as highly susceptible, marginally susceptible and non- susceptible in accordance with CLSI guidelines.

Screening for ESBL Activity

The ESBL screening was performed using the combined disc diffusion method following CLSI guidelines. Only carbapenem-sensitive isolates were selected for testing. A bacterial suspension was prepared in nutrient broth from overnight MacConkey agar cultures which and adjusted to a 0.5

McFarland standard (approximately 1.5 × 10^8 CFU/ml). Using the sterile cotton swab, the standardized suspension was uniformly spread over agar plates (Mueller–Hinton) to form a lawn culture. After allowing the surface to dry, discs containing 30µg cefotaxime and 30µg/10µg cefotaxime–clavulanic acid were positioned on the agar. Aerobic incubation was done at 35°C for 16 to 18 hours. An ≥5 mm increase in the zone around the cefotaxime–clavulanic acid disc versus cefotaxime disc alone was interpreted as indicative of ESBL production.

Screening for AmpC Expression

AmpC β-lactamase expression was screened in carbapenem-sensitive isolates using a cefoxitin (30 µg) disc, following CLSI guidelines. A bacterial suspension was prepared from colonies grown overnight on MacConkey agar which was equivalent to 0.5 McFarland turbidity. The suspension was uniformly spread on agar plates (Mueller–Hinton), which were left to dry before placing a cefoxitin (30 µg) disc at the center. Plates were incubated under aerobic condition at 35°C for 16 to 18 hrs, after which the inhibition was measured to assess potential AmpC activity.

Molecular Detection: Polymerase Chain Reaction (PCR)

DNA Extraction

For isolates confirmed as carbapenem-resistant, DNA extraction was performed. The Real Genomics DNA Extraction kit (version 2013-1) of RBC Bioscience Ltd was used, and bacterial isolate DNA was extracted using the alkaline lysis method.

Polymerase Chain Reaction Procedure

The DNA obtained was maintained at 4°C and later utilized for PCR to identify the blaNDM−1 gene. Prior to the procedure, the DNA was brought to room temperature. The PCR molecular technique for blaNDM−1 gene detection involved three primary steps: preparation of the reaction mixture, amplification in a thermocycler, and detection of the amplified product via gel electrophoresis.

Step A: Preparation of Reaction Mixture The reaction mixture comprised the following components: Dream Taq PCR master mix (2X) from Thermoscientific, NDM forward and reverse primers: F: 5'-GGTTTGGCGATCTGGTTTTC-3' and R: 5'-CGGAATGGCTCATCACGATC-3' located at gene positions 133-153 and 734-754 respectively [4], MiliQ water, DyNAzyme II DNA polymerase from Thermo Scientific , and the extracted DNA sample. The primers were designed based on the blaNDM−1 gene sequence available in the GenBank (accession number FN392876). The negative controls were the reference strains producing blaIMP, blaVIM, blaSPM, blaGIM, and blaSIM genes. The final total reaction mixture volume was 25 µl, with specific concentrations of each component as detailed in the table 1 below:

Table 1: Components and their respective volumes for the PCR reaction mixture, including negative and positive controls, and samples.

Reaction Components	**Negative Control (NC) in µl**	**Positive Control (PC) in µl**	**Sample in µl**
Master Mix	12.5	12.5	12.5
Forward primer	1	1	1
Reverse primer	1	1	1
Mili-Q water	10.5	8.5	8.5
Template	---	2	2
Total	**25**	**25**	**25**

The prepared reaction mixture was loaded into a thermocycler.

Step B: Amplification in Thermocycler Amplification was carried out according to the thermal cycling protocol below:

Table 2: PCR thermal cycling conditions.

Initial Temperature	**36 Cycles of Amplification**	**Final Extension**

94°C (10 min)	94°C (30 sec)	72°C (5 min)
	52°C (40 sec)	
	72°C (50 sec)	

The amplified product was stored at 4 °C until it was loaded onto the gel for electrophoresis.

Step C: Gel Electrophoresis for Detection of Amplified Product: 1% agarose gel was prepared to resolved the DNA fragments by electrophoresis at 70 V for 90 minutes using 100 ml of 1X TBE buffer (composed of 1.0 M Tris, 0.9 M boric acid, and 0.01 M EDTA). Gels were stained with ethidium bromide at a final concentration of 5 µg/ml (Hi-Media) for visualization under UV illumination. A 1 µl sample was loaded with 5 µl of bromophenol loading dye (BPB dye in 1:5 concentration). A Themoscientific ladder (1500bp-100bp range) was included in a well, alongside the negative and positive controls in their respective wells. After electrophoresis, the gel was placed in a Life Technologies 'E-Gel Imager' UV trans-illuminator. Visualization and recording of results were performed using 'Gel Capture' software.

Determination of MICs for C/T and C/Aby E-Test

The E-test strips method was employed for determining the MICs of C/T and C/A. Commercially sourced E gradient diffusion strips (range: 0.016–256 µg/ml) were used for each antibiotic combination. Pure bacterial colonies from overnight cultures were mixed in sterile saline (0.85% NaCl) to a turbidity equivalent to 0.5 McFarland standard. The Mueller-Hinton agar plates were uniformly inoculated using a sterile cotton swab to form an even bacterial lawn. After allowing approximately 15 minutes for the plates to dry, E-test strips were carefully kept onto the agar surface. MIC values were recorded after incubation at 37°C for 16–18 hours, at the point where the bacterial inhibition ellipse intersected the gradient strip.

Statistical Analysis

All analyses were performed in SPSS programme, version 17.0 (SPSS 111 Inc., Chicago, USA). Categorical data were compared by Chi-squared test and Fisher's exact test (two-sided). Statistical significance was considered to be less than 0.05 for all tests.

Results

Study Population Demographics

The study consisted of a total of 78 patients, with a male predominance (51, 65.4%) compared to females (27, 34.6%). The most common sample type collected was swab (43), followed by pus (17), urine (11), blood (4), and tracheal samples (3).

Table 1: Demographic details of the study population, showing the distribution of patients by gender and the number of various sample types collected.

Sample type	**Number**
blood	4
Swab	43
Pus	17
Urine	11
Tracheal	3

Gender	**No.**	**Percentage**
Male	51	65.4%
Female	27	34.6%

Overall Comparative Susceptibility

The susceptibility profiles against C/A and C/T was determined in 34 *E. coli* and 38 *K. pneumoniae* isolates.

- *E. coli*: C/A demonstrated superior activity against *E. coli* with 88.2% (30/34) susceptible isolates and an MIC range of 0.016 - 256. In contrast, C/T showed 79.4% (27/34) susceptibility with an MIC range of 0.038 - 256. The susceptibility difference between C/A and C/T for *E. coli* isolates was statistically significant (P = 0.004).
- *K. pneumoniae*: C/A exhibited higher susceptibility (57.9%, 22/38) compared to C/T (50.0%, 16/38). The MIC range for C/A was 0.016 – 256, while for C/T it was 0.025 - 256. This difference was observed to be statistically significant (*P* = 0.049).

Table 2: Comparative antibacterial activity of C/A and C/T against E. coli and K. pneumoniae isolates. The table shows the number of total isolates, number and percentage of sensitive isolates, and range of MIC of each combination of antibiotics, as well as the P-value for statistical significance of the difference in susceptibility. (*denotes statistically significant difference, P < 0.05).

	Total number	C/A		MIC Range	C/T		MIC Range	P value[#]
		Sensitive	%		Sensitive	%		
E.coli	34	30	88.2	0.016 - 256	27	79.4	0.038 - 256	0.004*
K. pneumoniae	38	22	57.9	0.016 – 256	16	50.0	0.025 - 256	0.049*

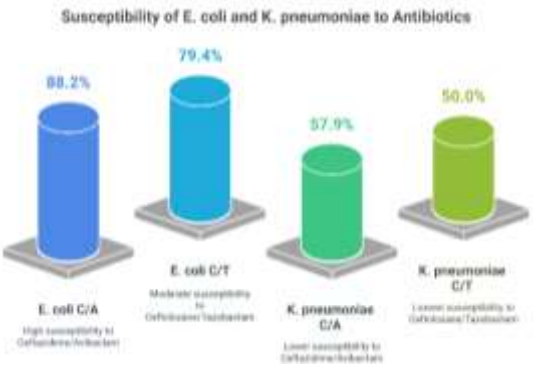

Figure 1. Comparative susceptibility profiles of *E. coli* and *K. pneumoniae* to C/A and C/T. The bar chart represents the percentages of susceptible isolates for each bacterial species, illustrating the relative in vitro effectiveness of these two antibiotic combinations.

Susceptibility by Resistance Phenotype

The antibacterial activity of C/A and C/T was further assessed in isolates categorized by ESBL, AMPc, and ESBL+AMPc phenotypes.

E. coli

- ESBL (n=10): The susceptibility was 100% among both C/A and C/T against ESBL-producing *E. coli* isolates, with MIC_{50}/MIC_{90} values of 0.023/1.864 for C/A and 0.22/3.9 for C/T; no statistical significant difference was found between these agents (*P* = 0.186).
- AMPc (n=6): C/A was susceptible in 50% (3/6) of AMPc-producing *E. coli* isolates (MIC50/MIC90: 128.32/256), while C/T showed 33.3% (2/6) susceptibility (MIC50/MIC90: 142.0/256). C/A demonstrated a statistically significant higher susceptibility rate against AMPc producers (P = 0.008).

- ESBL+AMPc (n=18): C/A showed 94.4% (17/18) susceptibility (MIC50/MIC90: 0.22/32.8), whereas C/T showed 83.3% (15/18) susceptibility (MIC50/MIC90: 0.875/256). However, **no statistical significant** difference (P = 0.156) was found.

K. pneumoniae

- ESBL (n=6): C/A showed 100% (6/6) susceptibility (MIC50/MIC90: 0.222/4.0), while C/T showed 83.3% (5/6) susceptibility (MIC50/MIC90: 0.315/32). No significant difference was observed (P = 0.234).
- AMPc (n=19): C/A demonstrated 52.6% (10/19) susceptibility (MIC50/MIC902.0/256) compared to C/T with 21.1% (4/19) susceptibility (MIC50/MIC90: 256/256); however, no **statistical significant** (*P* = 0.338) was found.
- ESBL+AMPc (n=7): C/A showed 85.7% (6/7) susceptibility (MIC50/MIC90: 0.19/256), while C/T showed 71.4% (5/7) susceptibility (MIC50/MIC90: 4.0/256). A statistical significant difference was found (*P* = 0.012).

Table 3. Comparative antibacterial efficacy of C/A and C/T against *E. coli* and *K. pneumoniae* isolates, categorized according to ESBL, AmpC, and combined ESBL+AmpC resistance phenotypes. Presented are MIC_{50}, MIC_{90}, MIC ranges, percentages of susceptible isolates (with sample sizes, n), and *p*-values for each comparison.

E. coli									
	C/A				C/T				
	MIC50	MIC90	MIC range	Susceptible (n) %	MIC50	MIC90	MIC range	Susceptible (n) %	p value
ESBL (10)	0.023	1.864	0.016-2.00	10 (100)	0.22	3.9	0.094-4.0	10 (100)	0.186
AMPc (6)	128.32	256	0.094-256	3 (50)	142.0	256	0.75-256	2 (33.3)	0.008
ESBL+AMPc (18)	0.22	32.8	0.016-256	17 (94.4)	0.875	256	0.038-256	15 (83.3)	0.156
K. pneumoniae									
ESBL (6)	0.222	4.0	0.016-4.0	6 (100)	0.315	32	0.19-32	5 (83.3)	0.234
AMPc (19)	2.0	256	0.064-256	10 (52.6)	256	256	0.25-256	4 (21.1)	0.338
ESBL+AMPc (7)	0.19	256	0.047-256	6 (85.7)	4.0	256	0.025-256	5 (71.4)	0.012

Overall Activity by Phenotype

Considering all isolates:

- ESBL (n=16): Both C/A (100% susceptible) and C/T (93.8% susceptible) demonstrated high activity, with C/A having MIC50/MIC90of 0.48/2.60 and C/T having 0.25/13.80.
- AMPc (n=25): C/A showed 52% (13/25) susceptibility (MIC50/MIC90: 2.00/256), which was significantly better than C/T's 24% (6/25) susceptibility (MIC50/MIC90: 256/256) (P = 0.007).
- ESBL+AMPc (n=25): C/A was susceptible in 92% (23/25) of cases (MIC50/MIC90: 0.19/107.2), while C/T was susceptible in 80% (20/25) of cases (MIC50/MIC90: 1.0/256). Statistical analysis revealed no significant difference (p = 0.269).
- NDM Negative (n=12): C/T showed significantly higher susceptibility (58.3%, 7/12) compared to C/A (8.3%, 1/12), with MIC50/MIC90of 7.0/256 for C/T and 256/256 for C/A (P = 0.377).

Table 4. Comparative antibacterial activity of C/A and C/T against bacterial isolates, categorized by ESBL, AmpC, and ESBL+AmpC resistance phenotypes. The table summarizes MIC50, MIC90, MIC range, susceptibility rates (% susceptible isolates, n), and corresponding *p*-values for each comparative analysis.

	C/A				C/T				P Value
	MIC50	MIC 90	Mic range	Susceptible (n)%	MIC50	MIC 90	Mic range	Susceptible (n)%	
ESBL (16)	0.48	2.60	0.016-4.0	16 (100)	0.25	13.80	0.94-32	15 (93.8)	-
AMPc (25)	2.00	256	0.064-256	13 (52)	256	256	0.25-256	6 (24)	0.007*
ESBL+AMPc (25)	0.19	107.2	0.016-256	23 (92)	1.0	256	0.025-256	20 (80)	0.269
NDM Negative (12)	256	256	5-256	1 (8.3)	7.0	256	0.25-256	7 (58.3)	0.377

Figure 2: Antibiotic Resistance patterns of Amikacin (AMK), Netilmicin (NET), Cefoperazone-Sulbactam (CFS), and Ciprofloxacin (Cipro) in *E. coli* and *K. pneumoniae* isolates, categorized by the presence of ESBL, AMPc (AmpC beta-lactamase), and ESBL+AMPc phenotypes.

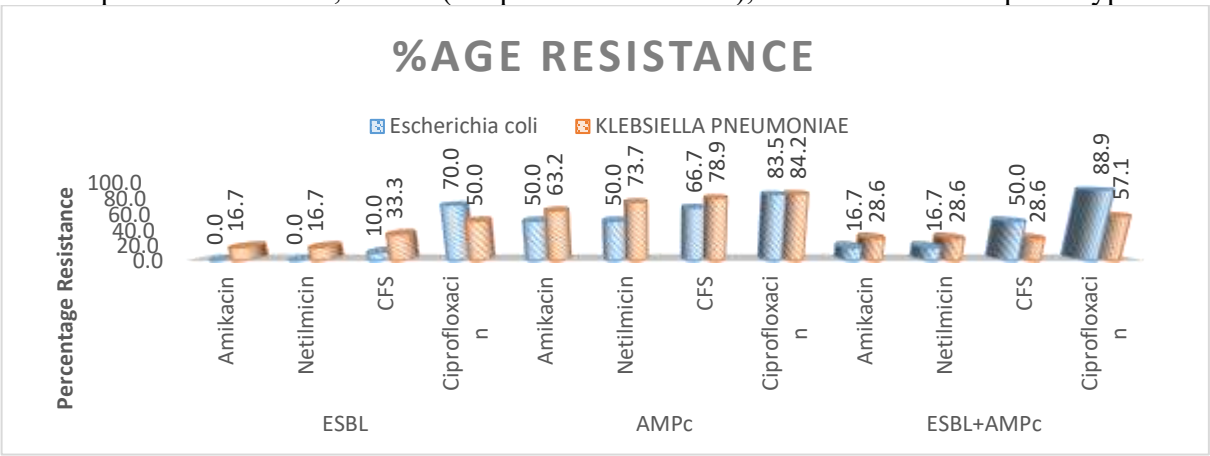

Table 5: Antibacterial efficacy of C/A and C/T against isolates exhibiting ESBL, AMPc, and ESBL+AMPc phenotypes. The table provides MIC50, MIC90, MIC range, and the percentage of susceptible isolates (n) for Amikacin, Netilmicin, Cefoperazone-Sulbactam (CFS), and Ciprofloxacin against each resistance phenotype.

ESBL								
	C/A				**C/T**			
	MIC50	**MIC90**	**MIC range**	**Susceptible (n)%**	MIC50	**MIC90**	**MIC range**	**Susceptible (n)%**
AMK	4	4	4-4	1 (100)	32	32	32-32	0 (0.0)
NET	4	4	4-4	1 (100)	32	32	32-32	0 (0.0)

CFS	0.94	3.8	0.069-4	3 (100)	0.25	29.2	0.19-32	2 (66.7)
Cipro	0.125	4	0.16-4	10 (100)	0.38	32	0.125-32	9 (90)
AMPc								
	C/A				**C/T**			
	MIC50	**MIC90**	**MIC range**	**Susceptible (n)%**	MIC50	**MIC90**	**Mic range**	**Susceptible (n)%**
AMK	256	256	0.19-256	6 (40)	256	256	0.25-256	1 (6.7)
NET	256	256	0.19-256	7 (41.2)	256	256	0.25-256	1 (5.9)
CFS	256	256	0.19-256	8 (42.1)	256	256	0.25-256	2 (10.5)
Cipro	256	256	0.125-256	10 (47.6)	256	256	0.25-256	4 (19)
ESBL+AMPc								
	C/A				**C/T**			
	MIC50	**MIC90**	**MIC range**	**Susceptible (n)%**	**MIC50**	**MIC90**	**MIC range**	**Susceptible (n)%**
AMK	0.75	256	0.020-256	4 (80)	6	256	0.125-256	3 (60)
NET	0.75	256	0.023-256	4 (80)	6	256	0.125-256	3 (60)
CFS	0.25	7.6	0.023-256	9 (81.8)	0.875	233.6	0.094-256	9 (81.8)
Cipro	0.19	256	0.016-256	19 (95)	3	256	0.038-256	16 (80)

C/A generally demonstrated superior antibacterial activity against *E. coli* and *K. pneumoniae* isolates as compared to C/T, particularly against AMPc-producing strains. While both antibiotics showed high efficacy against ESBL-producing isolates, C/A consistently provided higher susceptibility rates across several categories.

Discussion

The rising global burden of AMR in GNB producing ESBLs and AmpC β-lactamases is a severe public health emergency, represents a profound public health crises, leading to heightened morbidity and mortality [2,9]. This rising resistance calls for enhanced development and assessment of new antimicrobial agents to complement the few therapeutic options that have been made available for these complicated infections. This study assessed C/A and C/T susceptibility profiles *in vitro* in carbapenem-susceptible, ESBL and AmpC-producers of *E. coli* and *K. pneumonia*. The findings highlight important distinctions in their activity profiles, particularly concerning AmpC β-lactamase producers.

The study found that C/A exhibited superior overall activity against *E. coli* (88.2% susceptible) compared to C/T (79.4% susceptible) (P = 0.004). Similarly, against *K. pneumoniae*, C/A showed higher susceptibility (57.9%) than C/T (50.0%) (P = 0.049). When analyzed by phenotypic resistance profile, ESBL-producing *E. coli* isolates were 100% susceptible to both C/A and C/T. C/A showed significantly higher susceptibility against AmpC-producing *E. coli* (50% vs. 33.3%; P = 0.008) and AmpC-producing *K. pneumoniae* (52.6% vs. 21.1%; P = 0.338). For ESBL+AmpC co-producers, C/A showed 94.4% susceptibility against *E. coli* and 85.7% against *K. pneumoniae*, generally

outperforming C/T. Notably, C/T exhibited better activity against NDM-negative isolates (58.3% susceptible) than C/A (8.3% susceptible).

These findings align with existing literature indicating C/A's broader spectrum of activity, particularly against AmpC-producing isolates, due to avibactam's inhibitory effect on Class C β-lactamases. Studies have also noted that cefepime/tazobactam shows broad activity against AmpC and ESBL-producing *Enterobacterales*, attributing this to cefepime's inherent stability to these enzymes. Conversely, tazobactam's activity is more limited to Class A enzymes, which explains C/T's variable efficacy against AmpC producers [28]. The ESBL-producing *E. coli* showing high susceptibility of both C/A and C/T is consistent with numerous studies. A meta-analysis reported pooled ESBL-producing *E. coli* with 91.3% susceptibility rates for C/T and 65.6% for ESBL-producing *K. pneumoniae*. Supporting these findings, other studies have documented C/T susceptibility in 90.0% of ESBL-positive, carbapenemase-negative *E. coli* isolates, compared to 70.1% for *K. pneumoniae* [22, 30]. In contrast, the present study observed the ESBL-producing *E. coli* showing 100% susceptibility to both C/A and C/T, strongly suggesting the continued effectiveness of these agents against such resistant strains in the local setting.

The differential activity against NDM-negative isolates, where C/T showed higher susceptibility, is a notable exception. While C/A nor C/T neutralizes metallo-β-lactamases (MBLs) like NDM, the better performance of C/T in NDM-negative strains might be attributed to the intrinsic antibacterial potency of ceftolozane. This is further supported by studies that found both C/A and C/T were inactive against MBL-producing *K. pneumoniae* isolates [32]. Research has also shown that *K. pneumoniae* and *E. coli* isolates producing NDM exhibit resistance to ceftazidime–avibactam, limiting the efficacy of this agent against such strains. The prevalence of MBLs, particularly NDM, in India is significant, with 71% of isolates harboring the *blaNDM* gene. This underscores the critical need for molecular characterization to guide therapeutic decisions in regions with high MBL prevalence. The study also highlighted the importance of local susceptibility data. Some findings demonstrated that "Ceftriaxone-sulbactam-EDTA" (CSE) showed high sensitivity (95%) against ESBL and MBL-producing *Enterobacteriaceae* and non-*Enterobacteriaceae* groups, suggesting it as a potential carbapenem-sparing agent [31, 29, 33].

Overall, the findings of the study supports C/A as a potent agent against AmpC-producing *Enterobacteriaceae* and both agents as highly effective against ESBL producers. However, the varying efficacy against NDM-negative isolates and the universal lack of activity against MBLs emphasize the necessity for precise diagnostics and localized surveillance programs to inform targeted therapy and combat the evolving landscape of antimicrobial resistance.

Conclusion:

C/A generally demonstrated superior antibacterial activity against E. coli and *K. pneumoniae* isolates compared to C/T, particularly against AmpC-producing strains. Both agents proved highly effective against ESBL-producing isolates, with 100% susceptibility observed in ESBL-producing E. coli. Notably, C/A showed significantly higher susceptibility rates across various resistance categories, including AmpC-producing E. coli (50% vs. 33.3%) and K. pneumoniae (52.6% vs. 21.1%)18. However, C/T exhibited better activity against NDM-negative isolates (58.3% vs. 8.3%), highlighting a specific niche for its use. The findings underscore the importance of local surveillance data and molecular resistance mechanism characterization, particularly for endemic areas with prevalent MBL, for the optimization of empirical and specific regimens to treat MDR Gram-negative infections. The strategic incorporation of these next generation β-lactam/β-lactamase inhibitor combinations can be a part of carbapenem-sparing therapy and enhance patient outcomes in the presence of rising antimicrobial resistance.

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