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ORIGINAL ARTICLE

Evaluation of susceptibility to colistin, fosfomycin and mecillinam in carbapenemase-producing Enterobacterales isolated in Marrakech in 2023

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ABSTRACT

Background and Objectives: Carbapenemase-producing Enterobacterales (CPE) constitute a critical public health concern due to multidrug resistance and limited available treatment options. This study aimed to assess the in vitro susceptibility of CPE isolates to colistin, fosfomycin, and mecillinam.

Materials and Methods: Prospective study was conducted in 2023 at Mohammed VI University Hospital, Marrakech. A total sample of 180 non-duplicate CPE isolates were collected and identified by standard microbiological methods. Antimicrobial susceptibility testing was performed following recommended guidelines.

Results: Among the isolates, *Klebsiella pneumoniae* (60%) and *Escherichia coli* (14%) predominated. The most common carbapenemase was NDM (62%), followed by OXA-48 (26%) and co-producers (11%). Most isolates were obtained from intensive care (32%), plastic surgery (13%), and neonatology (12%) units. Skin and soft tissue (43%) and bloodstream (21%) were the predominant infected sites. Resistance rates were 25% for colistin, 48% for fosfomycin, and 64% for mecillinam, with frequent co-resistance to fluoroquinolones, aminoglycosides, and cotrimoxazole.

Conclusion: The high prevalence of NDM-producing *Klebsiella pneumoniae* and significant resistance to last-line agents suggest the urgent need for antimicrobial stewardship, optimized therapeutic strategies, and strengthened regional surveillance.

Keywords: Enterobacterales; Carbapenem-resistant Enterobacterales; Drug resistance; Bacterial; Colistin; Fosfomycin

INTRODUCTION

Carbapenemase-producing Enterobacterales (CPE) have emerged over the past decade as a significant global public health threat due to their ability to hydrolyze carbapenems, which are considered last-resort antibiotics used for treating severe infections caused by multidrug-resistant Gram-negative bacteria (1). Mobile genetic elements facilitate rapid dissemination of carbapenemase genes—such as KPC, NDM, VIM, OXA-48, and IMP—and their rapid global spread across hospital and community settings, raising serious concerns regarding infection control and antimicrobial stewardship (1, 2).

CPE infections are associated with significant clinical and economic burdens, such as increased mor-

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bidity and mortality rates, prolonged hospital stays, limited therapeutic options, and increased healthcare costs (2). These organisms frequently exhibit co-resistance to multiple classes of antibiotics, including fluoroquinolones, aminoglycosides, and sulfonamides, further complicating treatment strategies and reducing the effectiveness of conventional antimicrobial regimens.

In this context of growing antimicrobial resistance, there has been renewed interest in the use of older antibiotics such as colistin, fosfomycin, and mecillinam. Colistin (polymyxin E), despite its documented nephrotoxicity and neurotoxicity, has been reintroduced as a therapy for infections caused by extensively drug-resistant Gram-negative bacteria (3-5). Fosfomycin, a phosphonic acid derivative, exhibits broad-spectrum bactericidal activity and favorable pharmacokinetic properties, making it particularly useful for treating urinary tract infections and as part of combination therapies against multidrug-resistant pathogens (1). Mecillinam, a β-lactam antibiotic that belongs to the amidinopenicillin class, is mainly active against Enterobacterales and has shown effectiveness in the treatment of uncomplicated urinary tract infections, with potential utility against resistant strains.

In Morocco, recent studies have reported a concerning rise in the prevalence of CPE isolates, particularly those producing OXA-48 and NDM carbapenemases, reflecting regional and global epidemiological trends (5-7). However, data on the susceptibility of these strains to alternative therapeutic agents, such as colistin, fosfomycin, and mecillinam, remain limited.

Therefore, the present study aimed to characterize the epidemiological profile of carbapenemase-producing Enterobacterales isolated in 2023, assess their susceptibility to colistin, fosfomycin, and mecillinam, and evaluate the potential role of these antibiotics in the management of infections caused by these highly resistant pathogens.

MATERIALS AND METHODS

This prospective study was conducted at the microbiology laboratory of Mohammed VI University Hospital, Marrakech, between January and December 2023. Bacterial identification was performed using matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry. Briefly, isolated colonies were applied to a target plate, overlaid with a matrix solution, and analyzed using a MALDI-TOF mass spectrometer according to the manufacturer's instructions. Identification was based on comparison of the obtained spectra with the reference database.

Minimum inhibitory concentrations (MICs) were determined using the Phoenix M50® system. Carbapenemase types were identified with the NG-Test CARBA 5 assay (Fig. 1). Susceptibility to fosfomycin and mecillinam was assessed with disk diffusion (Fig. 2), while colistin susceptibility was evaluated by broth microdilution (Fig. 3), following EUCAST 2023 guidelines. Quality control strains were included.

In parallel, antimicrobial susceptibility testing of additional antibiotics was carried out by the disk diffusion method and interpreted according to EUCAST breakpoints.

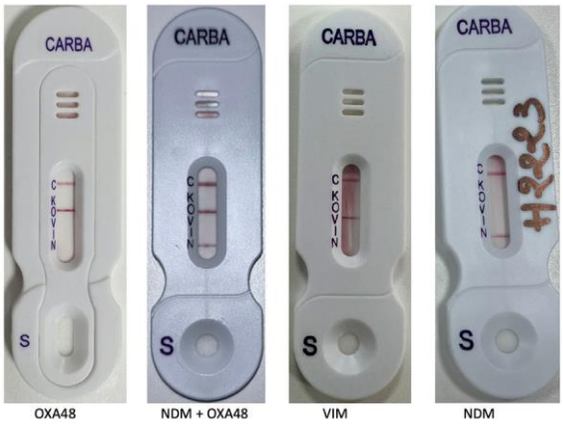

Fig. 1. Results of NG-Test CARBA-5 performed in the microbiology laboratory, illustrating the different carbapenemase types identified.

Fig. 2. Standard antibiogram performed on a *Klebsiella pneumoniae* isolate using fosfomycin and mecillinam disks.

Fig. 3. Example of a colistin plate used in the microbiology laboratory to study colistin susceptibility.

RESULTS

A total of 180 CPE isolates were analyzed. The most common species were *Klebsiella pneumoniae* (60%) and *Escherichia coli* (14%) (Table 1).

NDM carbapenemases were most frequent (62%), followed by OXA-48 (26%), and co-producers (11%) (Fig. 4).

The majority of isolates originated from intensive care (32%), plastic surgery (13%), and neonatology (12%). Skin and soft tissue infections (43%) and bloodstream infections (21%) predominated.

The susceptibility rates of the isolates varied according to the antibiotic tested. The highest susceptibility was observed for mecillinam (64%), followed by fosfomycin (48%), whereas the lowest susceptibility rate was recorded for colistin (25%), as shown in Table 2.

High co-resistance was observed with fluoroquinolones (90.3%), gentamicin (90%), and cotrimoxazole (90.6%). The reported high co-resistance rates correspond to isolates that were resistant to colistin, fosfomycin, and/or mecillinam and also showed resistance to fluoroquinolones (90.3%), gentamicin (90%), and cotrimoxazole (90.6%) as mentioned in Fig. 5.

Table 1. Distribution of isolated CPE according to bacterial species (n = 180)

Bacteria	Number	Percentage
Klebsiella pneumoniae	108	60%
Escherichia coli	25	14%
Enterobacter hormaechei	17	9%
Serratia marcescens	13	8%
Enterobacter cloacae	11	6%
Morganella morganii	2	1%
Klebsiella oxytoca	2	1%
Citrobacter freundii	2	1%
Total	180	100%

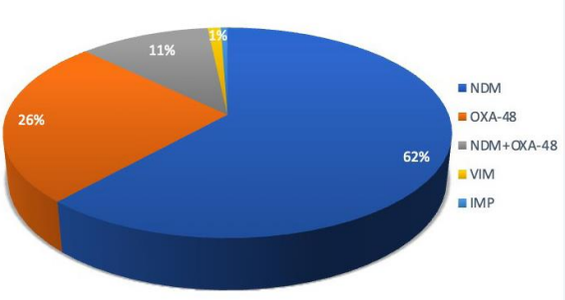

Fig. 4. Distribution of CPE according to the type of Carbapenemase identified (n = 180)

Table 2. Susceptibility profiles of isolates to colistin, fosfomycin, and mecillinam (n = 180).

Antibiotics	Susceptibility rate
Colistin	25%
Fosfomycin	48%
Mecillinam	64%

DISCUSSION

Our study highlights the epidemiological and resistance patterns of carbapenemase-producing Enterobacterales (CPE) found in Marrakech during 2023, providing valuable insights into a rapidly evolving public health problem. The predominance of *Klebsiella pneumoniae* and *Escherichia coli* among CPE isolates is also in line with national and international reports, as these species are the most efficient in acquiring and disseminating carbapenemase genes (5-10). Similar conclusions were reported in other Moroccan centers such as Rabat and Casablanca, and across North Africa, particularly in Tunisia and Algeria, where NDM- and OXA-48-type carbapenemases dominate the resistance landscape (5, 9). This reflects the transnational dissemination of these enzymes, largely facilitated by mobile genetic elements and inter-hospital patient transfers.

The predominance of NDM carbapenemases in our setting (62%) (Fig. 4) mirrors global trends observed in South Asia, the Middle East, and Africa, where NDM is endemic (5, 9).

OXA-48, initially described in Turkey, remains

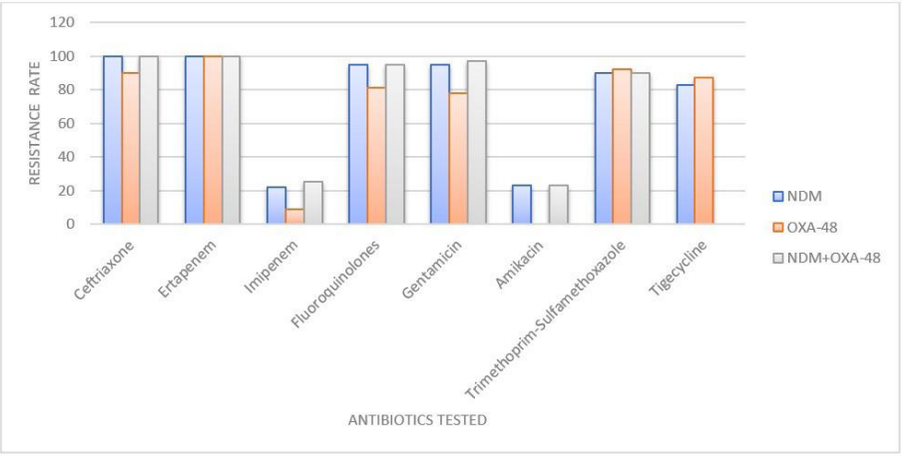

Fig. 5. Resistance percentage of CPE strains to antibiotics according to different carbapenemase.

widely disseminated in the Mediterranean basin and accounted for 26% of our isolates (5, 9). The co-production of NDM and OXA-48, observed in 11% of strains, is particularly concerning, as it confers resistance to nearly all β-lactams, complicating therapeutic options. The distribution of carbapenemases across hospital units further highlights risk environments: intensive care units (32%) are known hotspots due to high antibiotic pressure, invasive procedures, and vulnerable patient populations, while plastic surgery and neonatology units emphasize the diversity of clinical settings at risk (5, 6).

From a clinical standpoint, the high burden of skin and soft tissue infections (43%) and bloodstream infections (21%) among our cases is worrisome, as these presentations are associated with significant morbidity and mortality. Bloodstream infections caused by CPE have been repeatedly shown to carry mortality rates exceeding 30-40%, especially when appropriate therapy is delayed. Our findings align with multicenter studies in Europe and Asia reporting similar infection profiles in CPE outbreaks (9, 11, 12).

The resistance rates to colistin, fosfomycin, and mecillinam provide important insights into potential salvage therapies. Colistin resistance (25%) in our study is lower than rates reported in some European cohorts (>40%) but remains alarming considering its role as a last-resort antibiotic. The widespread use of colistin in empirical regimens has been linked to rapid emergence of resistance, often mediated by chromosomal mutations or transferable mcr genes (6, 13). The high proportion of colistin resistance among *K. pneumoniae* isolates (up to 73% in some subsets) is particularly problematic, as this species is responsible for most hospital-acquired infections (14, 15).

Fosfomycin resistance (48%) in our cohort is consistent with international studies, including those from China and Egypt, where resistance ranges from 40% to 60% (10, 16). While fosfomycin retains activity against certain urinary isolates, its efficacy in systemic infections is questionable due to pharmacokinetic limitations and rapid development of resistance under selective pressure. Nevertheless, fosfomycin has been shown to be effective in combination regimens, particularly when paired with colistin or carbapenem–β-lactamase inhibitor combinations, and may remain an important adjunct in salvage therapy (16).

Mecillinam resistance (64%) in our isolates underscores its limited role outside uncomplicated urinary tract infections. Although mecillinam has been repositioned in some European countries for extended-spectrum β-lactamase (ESBL)-producing Enterobacterales, its activity against CPE remains poor. Our results are in line with recent in vitro studies showing low efficacy of mecillinam against NDM- and OXA-48-producing strains (17-21). These findings suggest that reliance on mecillinam in our context would likely result in treatment failure for severe infections.

An additional concern is the very high co-resistance observed across other antibiotic classes, including fluoroquinolones (90.3%), gentamicin (90%), and cotrimoxazole (90.6%). This pattern leaves clinicians with extremely limited therapeutic options and highlights the potential for pan-resistant phenotypes (5, 7, 9). It also illustrates the collateral damage of

antibiotic misuse in both hospital and community settings, emphasizing the need for antimicrobial stewardship programs tailored to local epidemiology. The therapeutic implications of these findings are profound (22). In our setting, clinicians face an increasing number of infections caused by organisms resistant to nearly all available antibiotics. While colistin and fosfomycin may still play a role in selected cases, the high resistance rates observed demand caution (23). Newer agents such as ceftazidime-avibactam, meropenem-vaborbactam, and cefiderocol, though not widely available in Morocco, represent promising alternatives that must be evaluated in future studies. In the meantime, combination therapy remains the most pragmatic strategy, as supported by several observational studies demonstrating improved outcomes compared to monotherapy in CPE infections (24).

Beyond therapeutic considerations, our study underscores the importance of infection prevention and control (IPC). The clustering of cases in ICUs and surgical units highlights the need for strict hand hygiene, environmental cleaning, contact precautions, and antimicrobial restriction policies. Surveillance cultures and molecular typing could further clarify transmission dynamics and help contain outbreaks. Integration of our data into national AMR surveillance systems, such as GLASS, would provide policymakers with critical evidence to guide antibiotic procurement and stewardship strategies.

Finally, this study has limitations. Being monocentric, the results may not be fully generalizable to other Moroccan regions. The absence of molecular sequencing limits our understanding of clonal relatedness and the specific resistance genes involved. Despite these limitations, our prospective design, use of reference methods (MALDI-TOF, broth microdilution, NG-Test CARBA 5), and relatively large sample size provide robust evidence of the alarming resistance landscape in Marrakech.

In conclusion, our results confirm that CPE represent a critical public health threat in Morocco, characterized by high prevalence of NDM and OXA-48, significant resistance to last-resort antibiotics, and extensive co-resistance across multiple classes. Addressing this crisis requires a multifaceted approach combining antimicrobial stewardship, IPC, access to novel therapeutics, and regional collaboration. Without urgent action, the therapeutic void for severe Gram-negative infections will only deepen.

CONCLUSION

CPE infections present a considerable therapeutic challenge due to extensive multidrug resistance. Our study documents high prevalence of NDM- and OXA-48-producing strains in Marrakech, with concerning resistance to colistin, fosfomycin, and mecillinam. These findings reinforce the urgency of antimicrobial stewardship, strengthened infection control measures, and exploration of alternative therapeutic options. National surveillance and regional collaborations are essential to curb the spread of these pathogens and preserve the few remaining treatment options.

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