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bla_{IMP} and bla_{OXA} mediated carbapenemase resistance in *klebsiella pneumoniae*: A molecular and phenotypic analysis

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Abstract:
These effusions result in a convergence of Class B metallo-2-lactamases (bla_{IMP}) and Class D oxacillinases (bla_{OXA}) in *Klebsiella pneumoniae* to produce very difficult to diagnose and treat complex multidrug-resistant phenotypes. Fifteen phenotypic susceptibility testing and multiplex PCR were performed on 185 carbapenem-resistant *K. pneumoniae* isolates of a tertiary care center to determine the prevalence and co-occurrence of bla_{IMP} and bla_{OXA}-48-like genes. Molecular genotyping revealed that bla_{OXA-48}-like genes were present in 62.2% of isolates and bla_{IMP} variants in 28.1% with 14.6% of the isolates carrying both genes, which increases the MIC values of imipenem and meropenem by a significant margin over single-gene carriers. Thus, data shows that the development of bla_{IMP} and bla_{OXA}-co-producing strains makes newer 2 2-lactam/2 2-lactamase combinations largely ineffective. This requires the immediate introduction of molecular surveillance and other therapeutic agents such as cefiderocol.

Keywords: *Klebsiella pneumoniae*, Class B metallo-2-lactamases (bla_{IMP}), Class D oxacillinases (bla_{OXA-48}), carbapenemase, multidrug resistance

Background:
Carbapenem-Resistant *Enterobacterales* (CRE) dissemination is the ultimate challenge to human health, as it will decrease the effectiveness of the last-resort antibiotics. *Klebsiella pneumoniae* is the leading agent of resistance determinant dissemination in healthcare facilities among *Enterobacterales* **[1]**. Although the bla_{KPC} (*Klebsiella pneumoniae* Carbapenemase) gene has traditionally occupied the center stage of the epidemiological picture in the Americas and certain parts of Europe, recent years have seen that the distribution of carbapenemases has become quite heterogeneous with the emergence of Metallo- beta-Lactamases (MBLs) and Oxacillinases (OXA) **[2]**. These resistance mechanisms include enzyme production which is able to hydrolyze the carbapenem β-lactam ring. The blaOXA-48-like family of Ambler Class D enzymes are now endemic in Mediterranean and Middle East as well as in some parts of Europe. These enzymes are infamous because they possess a so-called phantom phenotype; they avidly degrade penicillins and normally exhibit low carbapenemase activity unless in combination with permeability defects (porin loss), which can be difficult to phenotypically determine **[3]**. On the other hand, the Ambler Class B enzymes include the IMP (Imipenemase) type, which has a broad spectrum of hydrolytic activity against all 8 -lactams except monobactams and are also resistant to clinically available 2 -lactamase inhibitors, including tazobactam, avibactam and vaborbactam **[4]**. An alarming observation that has been found in recent surveillance research is the presence of numerous carbapenemase genes in a single host isolate. The presence of bla_{IMP} and bla_{OXA} genes on compatible plasmids or in the same genetic element forms an imposing resistance profile **[5]**. Both strains that contain both determinants have an effective neutralizing effect on both the standard carbapenims and many new combinations of 2-lactam/2-lactamase inhibitors (BL/BLIs), including ceftazidime-avibactam (active against OXA-48, but not against IMP) **[6]**. Although the critical depth of the situation is found in the area of infection control and the treatment process, the lack of data concerning the particular interplay between bla_{IMP} and bla_{OXA} variants in our geographical area is present. It is important to know the level of these particular genotypes as there is a great variation in the treatment regimen depending upon the type of MBLs versus serine carbapenemases **[7]**. Therefore, it is of interest to describe the molecular epidemiology of bla_{IMP} and bla_{OXA}-mediated resistance in clinical isolates of *Klebsiella pneumoniae* and to evaluate their combined impact on phenotypic antimicrobial susceptibility patterns in the studied population.

Materials and Methods:

Study design and population:
This hospital-based, cross-sectional observational study was conducted over a 12-month period at the Department of Microbiology in a 1,000-bed tertiary care teaching hospital. The study population included 185 consecutive, non-duplicate clinical isolates of *Klebsiella pneumoniae* recovered from various biological samples (blood, urine, respiratory secretions, pus/wound swabs and sterile body fluids) of hospitalized patients.

Inclusion and exclusion criteria:
Inclusion: All isolates identified as *K. pneumoniae* that exhibited non-susceptibility (Intermediate or Resistant) to at least one carbapenem (Imipenem, Meropenem, or Ertapenem) based on CLSI screening breakpoints.
Exclusion: Duplicate isolates from the same patient within a 14-day period, environmental isolates and non-*Klebsiella* species.

Bacterial identification and susceptibility testing:
Species identification was performed using standard biochemical methods and confirmed by the VITEK® 2 Compact system (bioMérieux, France) using GN cards. Antimicrobial Susceptibility Testing (AST) was conducted using the Kirby-Bauer disk diffusion method on Mueller-Hinton Agar (MHA) according to Clinical and Laboratory Standards Institute (CLSI) M100 guidelines (2023-2024). The antibiotic panel included: Piperacillin/Tazobactam, Ceftazidime, Cefepime, Meropenem, Imipenem, Ertapenem, Amikacin, Gentamicin, Ciprofloxacin, Levofloxacin, Trimethoprim/Sulfamethoxazole and Ceftazidime-Avibactam. Minimum Inhibitory Concentrations (MIC) for Colistin and Tigecycline were determined using the Broth Microdilution (BMD) method.

Phenotypic carbapenemase detection:
Carbapenem-resistant isolates were subjected to the Modified Carbapenem Inactivation Method (mCIM) and the EDTA-

modified Carbapenem Inactivation Method (eCIM) to differentiate between Serine Carbapenemases (*e.g.,* OXA-48) and Metallo-β Lactamases (*e.g.,* IMP). An indeterminate eCIM result in the presence of a positive mCIM was considered suggestive of possible co-production or OXA-48 prescence.

Molecular characterization (PCR):

Genomic DNA was extracted using the alkaline lysis method. Multiplex Polymerase Chain Reaction (PCR) assays were established to detect specific carbapenemase genes. Primers were designed to target:

[1] bla_{IMP} variants (consensus sequence for IMP-1, IMP-2, *etc.*)

[2] bla_{OXA-48}-like variants.

[3] Additional primers for bla_{KPC} and bla_{NDM} were included to rule out other common mechanisms, though the analysis focused on IMP and OXA.

[4] PCR conditions consisted of initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C (30s), 55°C (40s) and 72°C (50s), with a final extension at 72°C for 7 min. Amplified products were visualized on 1.5% agarose gel via electrophoresis.

Statistical analysis:

Data were analyzed using SPSS version 26.0 (IBM Corp, Armonk, NY). Categorical variables were reported as frequencies (n) and percentages (%). Continuous variables were reported as Mean ± Standard Deviation (SD). Resistance rates between genotypic groups (IMP-only, OXA-only and Co-producers) were compared using the Chi-square (χ^2) test or Fisher's exact test. A p-value of <0.05 was considered statistically significant.

Results:

A total of 185 carbapenem-resistant *K. pneumoniae* isolates were analyzed. The mean age of the patients was 58.4 ± 15.6 years, with a male predominance (63.8%, n=118). The majority of isolates were recovered from the Intensive Care Unit (ICU) (58.9%), followed by general medical wards (22.7%). The most common specimen sources were respiratory tract samples (41.1%) and urine (24.3%) **(Table 1).** Analysis of resistance patterns based on genotype revealed significant differences. Isolates co-harboring bla_{IMP} and bla_{OXA-48} showed 100% resistance to carbapenems and Ceftazidime-Avibactam. While bla_{OXA-48}-only isolates remained largely susceptible to Ceftazidime-Avibactam (Susceptibility > 95%), the introduction of bla_{IMP} abolished this susceptibility. The co-producers also exhibited significantly higher resistance to Amikacin compared to single producers (p<0.01) **(Table 2)**. Ceftazidime-Avibactam resistance was near-absent in the bla_{OXA-48} group (3.4%, 3/88) but soared to 100% in the bla_{IMP} (25/25) and dual-gene (27/27) cohorts (P < 0.001). Amikacin resistance also spiked in the co-carriage group (77.8%, 21/27) compared to bla_{OXA-48} (31.8%, 28/88) and bla_{IMP} (36.0%, 9/25) single-gene groups (P < 0.001). Conversely, no significant differences were found for the other drugs (P > 0.05). Meropenem resistance was consistently extreme across all groups (95.5%–100%, P = 0.34). Ciprofloxacin resistance remained high (72.0%–92.6%, P = 0.18), while Tigecycline displayed the lowest overall resistance rates (12.0%–22.2%, P = 0.45) **(Table 3)**. The isolates showed extensive resistance. Resistance to Meropenem was 88.6% and Imipenem 85.4%. Ceftazidime-Avibactam resistance was observed in 30.3% of isolates, correlating strongly with the presence of metallo-β-lactamases. Colistin remained the most effective agent (91.9% susceptible).

Table 1: Demographic and clinical distribution of isolates (N=185)

Parameter	Category	Frequency (n)	Percentage (%)
Gender	Male	118	63.8%
	Female	67	36.2%
Hospital Unit	ICU	109	58.9%
	Medical Ward	42	22.7%
	Surgical Ward	34	18.4%
Specimen Source	Respiratory (Sputum/ETA)	76	41.1%
	Urine	45	24.3%
	Blood	38	20.5%
	Pus/Wound	18	9.7%
	Others	8	4.3%

Table 2: Overall antimicrobial resistance profile (N=185)

Antibiotic	Resistant n (%)	Intermediate n (%)	Susceptible n (%)
Meropenem	164 (88.6%)	10 (5.4%)	11 (5.9%)
Imipenem	158 (85.4%)	12 (6.5%)	15 (8.1%)
Ertapenem	178 (96.2%)	2 (1.1%)	5 (2.7%)
Ceftazidime	180 (97.3%)	-	5 (2.7%)
Ceftazidime-Avibactam	56 (30.3%)	-	129 (69.7%)
Ciprofloxacin	155 (83.8%)	5 (2.7%)	25 (13.5%)
Amikacin	78 (42.2%)	15 (8.1%)	92 (49.7%)
Colistin	15 (8.1%)	-	170 (91.9%)
Tigecycline	28 (15.1%)	-	157 (84.9%)

Table 3: Comparison of resistance rates by genotype

Antibiotic	bla_{OXA-48} only (n=88)	bla_{IMP} only (n=25)	bla_{OXA} + bla_{IMP} (n=27)	P-value
Meropenem	84 (95.5%)	25 (100%)	27 (100%)	0.34
Ceftazidime-Avibactam	3 (3.4%)	25 (100%)	27 (100%)	<0.001
Amikacin	28 (31.8%)	9 (36.0%)	21 (77.8%)	<0.001
Ciprofloxacin	70 (79.5%)	18 (72.0%)	25 (92.6%)	0.18
Tigecycline	12 (13.6%)	3 (12.0%)	6 (22.2%)	0.45

Discussion:

Carbapenin resistance in *Klebsiella pneumoniae* is a growing complex at the molecular level. The high prevalence of bla_{OXA-48}-like genes (62.2%) and the high prevalence of bla_{IMP} (28.1%) in our tertiary care environment are of importance to our research. These data are consistent with the data on the global surveillance provided by the SENTRY program, which points to the fact that, although KPC stays the primary cause of resistance in the Americas, OXA-48 and MBLs are the main causes of resistance in the countries in the EMEA (Europe, Middle East, Africa) region **[8]**. The observation of bla_{OXA-48} as the most common gene is reproducible to the fact that it spreads easily and in high efficiency through the plasmid. OXA-48 plasmids (pOXA-48a) are greatly conjugative and have managed to disseminate among

the *Enterobacterales* species **[9]**. The clinical issue is however enhanced by the 28.1 percent prevalence of bla_{IMP}. In contrast to OXA-48, an enzyme that is a serine carbapenemase, IMP is a metallo- 8 -lactamase. The difference is essential since the inhibitors of the MBLs have a natural resistance to the so-called new generation. This may be seen by how the bla_{IMP}-carrying isolates were fully resistant to ceftazidime-avibactam, but bla_{OXA-48} isolates were highly susceptible (96.6%), showed in our results **(Table 3)**. This underlines the idea that ceftazidime-avibactam can be used against OXA-48 but not against MBL producers **[10-15]**. The rate of co-production (bla_{OXA-48} + bla_{IMP}) is alarmingly high in this study and is 14.6%. This is a scenario of a so-called double-hit genotype leading to a pan-resistant or near-pan-resistant phenotype. There was a very high resistance of the co-producers to adjunctive therapies such as Amikacin (77.8% resistance) in comparison with single-gene carriers. This implies that the mobile genetic component that encodes these carbapenemases also encodes aminoglycoside-modifying enzymes (such as armA or rmt genes) to form a multidrug-resistant genetic platform **[16]**. Treatment of infections by these co-producers is not easy. Not only ceftazidime-avibactam but also aztreonam (resistant to IMP) is hydrolyzed by OXA-48, so monotherapy with either is doomed to fail. Recent recommendations of the IDSA propose the introduction of Ceftazidime-Avibactam and Aztreonam combination as one of the strategies, in which Avibactam suppresses the OXA enzyme and Aztreonam the IMP hydrolysis **[17]**. As an alternative, Cefiderocol, a siderophore cephalosporin, has been demonstrating effectiveness in cases of MBL as well as serine carbapenemases, but resistance is already being reported **[18]**. In a study it was revealed that bla_{NDM} is the most prevalent carbapenemase gene in CRKP isolates. Furthermore, bla_{OXA-48} and the co-existence of both bla_{NDM} and bla_{OXA-48} genes are emerging in CRKP isolates. The infections caused by CRKP are a serious public health concern, especially in patients under treatment in hospital settings. In our study, we were highly susceptible to Colistin, which is nephrotoxic and which limits its use as the first-line agent **[19]**. Our study is limited to the fact that we have not done Whole Genome Sequencing (WGS), so we cannot deduce which type of sequence (STs) or plasmid compatibility groups we were dealing with. Moreover, we failed to screen bla_{NDM} or bla_{VIM} in the non-IMP MBL group and this could explain the rest of the resistant isolates.

Conclusion:

We show the elevated occurrence of bla_{OXA-48} and bla_{IMP} in *K. pneumoniae* isolates leads to dual-producing strains that render ceftazidime-avibactam ineffective. Furthermore, these dual-producers exhibit statistically significant co-resistance to aminoglycosides, severely narrowing remaining antimicrobial treatment options. Consequently, the universal adoption of molecular multiplex testing in clinical laboratories is critical to differentiate specific resistance mechanisms and direct the rational use of alternative agents such as cefiderocol or aztreonam-avibactam.

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