Evaluation of Ceftazidime-Avibactam resistance in clinical isolates of Enterobacterales from a large tertiary-care hospital, Italy, 2019-2023

POSTER No: P024

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ABSTRACT

Introduction

Over the past decades, carbapenemase-resistant Enterobacterales (CRE), notably KPC-producing *K. pneumoniae*, have spread in health care facilities and have become a serious global threat. Among the new β-lactam/β-lactamase-inhibitors combinations, ceftazidime-avibactam (CZA) was the first approved for the treatment of infections caused KPC-producing strains. Despite its recent commercialization, an increasing number of cases of CZA-resistant (CZAr) CRE have been reported. The aim of this study is to investigate the molecular bases of CZA resistance in a collection of CRE of clinical origin collected at the San Martino Hospital (Genoa) from 2019 to 2023.

Materials and methods

Identification and antibiotic susceptibility testing were performed with Vitek MS MALDI-TOF and VITEK2 system, respectively. Carbapenemases production (KPC, VIM, NDM and OXA-48 like enzymes) was assessed with immunochromatographic assays and qualitative RT-PCR. Resistance to CZA and meropenem (MEM) was confirmed by reference broth microdilution method and MICs were interpreted according to EUCAST v13.0 clinical breakpoints. The bla_{KPC} gene copy-number was determined by relative RT-PCR. CZAr strains were subjected to WGS.

Results

We studied 71 CZAr isolates (31 *K. pneumoniae*, 21 *E. cloacae cpx*, 2 *S. marcescens*, 5 *K. oxytoca*, 4 *E. coli* e 7 *C. freundii*, 1 *C. portucalensis*), of which 68% (48/71) tested positive for VIM, 18% (13/71) for KPC, 8% (6/71) for KPC and VIM and 6% (4/71) for NDM. In CZAr-KPC producing strains (13 *K. pneumoniae*), MIC values ranged from 16 to 128 mg/L for CZA and from 0,5 to 512 mg/L for MEM. Among these, 77% (10/13 *K. pneumoniae*) were resistant to MEM, while 23% (3/13) (3 *K. pneumoniae*) tested susceptible. According to RT-PCR, 69% (9/13) of KPC-producing isolates showed an increased bla_{KPC} copy number. WGS results confirmed that the former CZAr isolates carried a bla_{KPC-3} gene, 8% (1/13) carried a bla_{KPC-3} with a non-functional *OmpK36* porin and 23% (3/13) carried a mutated bla_{KPC}, coding for *KPC-49*, *KPC-66*, *KPC-109*.

Discussion

Overall, present results showed that: i) in our setting, CZAr was largely associated with production of metallo-β-lactamase (mostly of VIM type); ii) among KPC-producing strains, CZAr was primarily associated with an increased bla_{KPC} copy number and to emergence of novel KPC variants, to a lesser extent. Present results highlight that overproduction of KPC could represent an important cause of CZAr. This genotype circumvents rapid molecular diagnostic tests, jeopardizing the inference of the putative resistance phenotype from rapid molecular tests and placement of appropriate antibiotic therapy.

INTRODUCTION

Carbapenemase-resistant Enterobacterales (CRE), notably KPC-producing *K. pneumoniae*, have spread in health care facilities and become a serious global threat. The new β-lactam/β-lactamase-inhibitors combinations, ceftazidime-avibactam (CZA) was the first approved for the treatment of infections caused KPC-producing strains[1], but however the recent commercialization, an increasing number of cases of CZA-resistant (CZAr) CRE have been reported[2].

This study investigate the molecular bases of CZA resistance in a clinical collection of CRE at the San Martino Hospital (Genoa) from 2019 to 2023.

MATERIALS AND METHODS

The methodological approach empolyed in this study included:

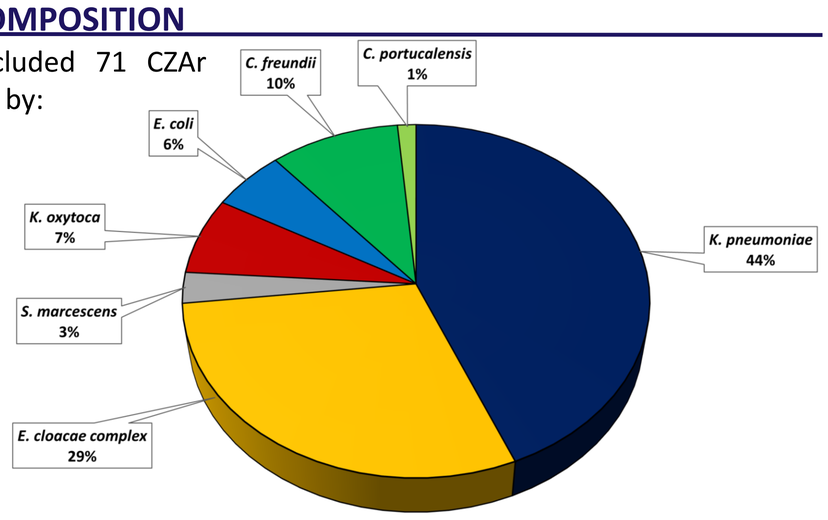
- Vitek MS MALDI-TOF and VITEK2 system for strains identification and antibiotic susceptibility testing, respectively[4];
- Immunochromatographic assays and qualitative RT-PCR to detect carbapenemases[4];
- Broth microdilution method to confirm resistance to ceftazidime-avibactam and meropenem[4];
- Quantitative RT-PCR to determine the bla_{KPC} gene copy-number[3];
- Sanger sequencing of PCR products to investigate novel bla_{KPC} allelic variants[4];
- Whole genome sequencing (WGS) to explore the genetic features of CZAr strains[5];

RESULTS

1 STARTING CLUSTER COMPOSITION

The study population included 71 CZAr isolates and was composed by:

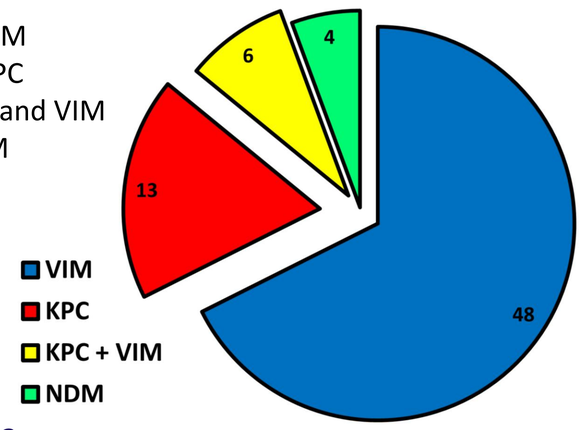
- 31 *K. pneumoniae;*
- 21 *E. cloacae cpx;*
- 2 *S. marcescens;*
- 5 *K. oxytoca;*
- 4 *E. coli;*
- 7 *C. freundii;*
- 1 *C. portucalensis*



2 DETECTION OF CARBAPENMASE

Investigation of either carbapenemase production or detection of carbapenemase-encoding genes revealed:

- 48/71 isolates (68%) tested positive for VIM
- 13/71 isolates (18%) tested positive for KPC
- 6/71 isolates (8%) tested positive for KPC and VIM
- 4/71 isolates (6%) tested positive for NDM



3 ANTIMICROBIAL SUSCEPTIBILITY TESTING

Focusing the study on CZAr KPC producing *K. pneumoniae* strains (n = 13*)*, AST yielded MIC values ranging from 16 to 128 mg/L for CZA and from 0.5 to 512 mg/L for MEM.

Among these:

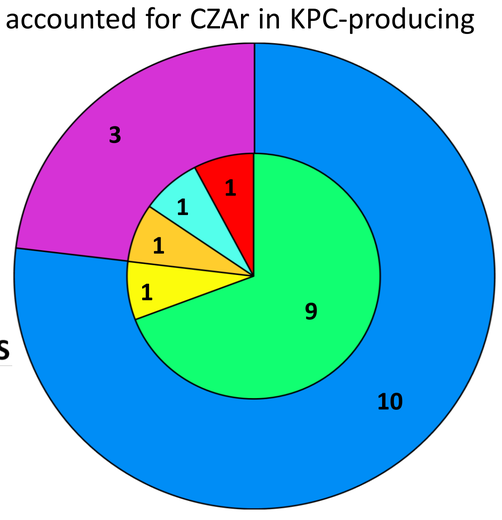
- 10/13 isolates (77%) tested were resistant to MEM;
- 3/13 isolates (23%) tested was susceptible to MEM;

		Strain n°												
		1	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***11***	***12***	***13***
MIC (mg/L)	***MEM***	>256	512	256	128	256	512	>256	256	>256	>256	1	4	4
	CZA	64	64	32	16	16	16	16	16	16	32	32	32	256

4 CZAr KPC-PRODUCING *K. pneumoniae*

According to RT-PCR and WGS, different mechanisms accounted for CZAr in KPC-producing isolates:

- 9/13 isolates showed an increased bla_{KPC} copy number (mean fold 6.2 ± 2.5);
- 3/13 isolates produced mutated KPC enzymes, including KPC-49, KPC-66, KPC-109[5];
- 1/13 strain produce a non-functional *OmpK36*[6] porin and showed an increased classic KPC-3 copy number



CONCLUSIONS

Overall, present results show that:

- In our setting, resistance to ceftazidime-avibactam was largely associated with production of metallo-β-lactamase (mostly of VIM type);
- Among KPC-producing strains, CZAr was primarily associated with an increased bla_{KPC} copy number, leading to overproduction of KPC, and to emergence of novel KPC variants.
- Overproduction of KPC or emergence of novel KPC variants associated with CZAr represent a major concern, because this genotypes hinder an effective recognition and inference of the associated phenotypes by rapid molecular tests (e.g. LFIA, RT-PCR).
- Surveillance studies are critically important to monitor the emergence of new resistance features in CPE, thus avoid delays in the application of appropriate antibiotic therapy.

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