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The threat of carbapenem-resistant hypervirulent *Klebsiella pneumoniae* (CR-HvKP).

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Abstract

Klebsiella pneumoniae **is one of clinically important opportunistic pathogens responsible for community-acquired and nosocomial infections in immunocompromised individuals. The increasing prevalence of carbapenem-resistant *K. pneumoniae* (CRKP) poses a serious threat to public health worldwide. With antibiotic resistance, hypervirulent *K. pneumoniae* (HvKP) responsible for serious disseminated infections in both healthy and immunocompromised individuals has also emerged. Recently, the emergence of carbapenem-resistant hypervirulent *K. pneumoniae* (CR-HvKP) strains has become a great challenge to public health. In this paper, we discuss the emergence mechanisms of CR-HvKP through analyzing features of CR-HvKP strains isolated in China, where both CRKP and HvKP strains are prevalent. These analyses suggest that more extensive surveillance is required to accurately assess the potential threat of the plasmid-mediated convergence of carbapenem-resistance and hypervirulence.**

Keywords: *Klebsiella pneumoniae*, Carbapenem-resistance, Hypervirulence, Plasmid-mediated convergence.

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Introduction

Antibiotic resistance is a serious public health crisis [1,2]. The gradual increase of carbapenemase-resistant (CR) *Klebsiella pneumoniae* is especially serious [3,4]. Several carbapenemases, such as *K. pneumoniae* Carbapenemases (KPC), carbapenemases of the oxacillinase-48 (OXA-48), and New Delhi metallo-β-lactamase (NDM) carbapenemases type, have been reported worldwide in *K. pneumoniae* [3-5]. Besides carbapenem resistance, another big problem of *K. pneumoniae* infection is the emergence of hypervirulent *K. pneumoniae* (HvKP) with hypervirulence [6-8]. This pathogen with the hypermucoviscous phenotype caused highly invasive infections, such as liver abscesses, in both healthy and immunocompromised individuals [7-9]. Unlike the classic *K. pneumoniae* (cKP), approximately half of all HvKP infections occur in young, healthy individuals [6,9]. Many reports have determined hypervirulence-associated factors, including a K1/K2 capsular serotype, ST23 sequence type, a pLVPK virulence plasmid, a KPHP1208 pathogenicity island, and several virulence factors, such as RmpA and aerobactin [6].

Antibiotic resistance of cKP is significantly high worldwide [3], but HvKP is rarely resistant to antibiotics, except for an intrinsic resistance to ampicillin [6,10]. However, recent many reports have shown the emergence of antibiotic-resistant HvKP strains [6]. Especially, the emergence of Carbapenem-Resistant HvKP (CR-HvKP) was reported in China, where both carbapenem-resistant cKP (CRKP) and carbapenem-susceptible HvKP strains are prevalent [6,11]. A recent study performed by Danxia et al. showed the emergence of CR-HvKP ST11 strains caused by the transfer of a 170 kb pLVPK-like virulence plasmid into bla_{KPC-2}-harboring ST11 CRKP stains [12]. The report provided a direct clue to plasmid-mediated convergence of carbapenem-resistance and hypervirulence. To understand the emergence mechanism of CR-HvKP, we analysed all reported CR-HvKP isolates. The analysis results suggested the following important aspects: (i) another possible mechanism of plasmid-mediated gene spread (the horizontal transfer of resistance plasmids from CRKP into HvKP strains); (ii) requirement for conjugation experiment of plasmids between HvKP and CRKP.

The Analysis of all Reported CR-HvKP Strains

Before the study of Danxia et al., there have been 36 CR-HvKP strains isolated in China between 2010 and 2018 [13-18]. Sequence types and genotypes of β-lactamase (*bla*) genes of 36 strains were summarized in Table 1. Notably, 34

(94%) of 36 strains have bla_{KPC-2} and 18 (50%) belong to ST11. Because the predominant clone of CRKP is KPC-2-producing ST11 and the dominant clone of HvKP is ST23 in China [6,11,19-22], these investigations imply the possibility for the direct transmission of a virulence plasmid from HvKP to KPC-2-producing CRKP. Danxia et al. proved this possibility.

Interestingly, investigation of 36 CR-HvKP isolates in China suggests another possible mechanism of plasmid-mediated gene spread. ST23 is the predominant clone of HvKP strains with the K1 serotype and ST65 is one of the dominant clones of HvKP with the K2 serotype [6,23]. These sequence types were rarely found in CRKP strains [3]. Notably, 9 (25%) of 36 CR-HvKP strains belonged to ST65 or ST23 (Table 1). Like the case of ST11, these imply the possibility for the direct transmission of a resistance plasmid carrying bla_{KPC-2} from CRKP to ST23 or ST65 HvKP strains. Because the first possibility (the transmission of a pLVP-like plasmid from HvKP to CRKP) based on sequence types of 36 CR-HvKP strains was proved, another possible mechanism (the transmission of the resistance plasmid from CRKP to HvKP) also should be investigated.

Danxia et al. showed that resistance plasmids harboring the bla_{KPC-2} gene of 5 CR-HvKP ST11 strains could be transferred to the *Escherichia coli* strain through conjugation experiments, but they did not examine whether the pLVPK-like plasmid of CR-HvKP strains can be transferred to the *E. coli* or *K. pneumoniae* strain through conjugation. Strictly speaking, it is necessary to check whether pLVPK-like plasmids of HvKP can be transferred to CRKP ST11 strains. These experiments will provide data supporting Danxia et al.'s finding, the acquisition of the pLVPK-like plasmid by CRKP ST11 strains. Like Danxia et al.'s conjugation experiments, a study in 2014 showed the transmission and retainment of a resistance plasmid carrying bla_{KPC-2} from a classical ST258 *K. pneumoniae* to a recipient hypervirulent ST65 *K. pneumoniae* strain through conjugation [24]. These results raise the possibility for the horizontal transfer of resistance plasmids of CRKP into hypervirulent *K. pneumoniae* strains. When Danxia et al.'s study is included, the rate (56%, 23 of 41) of ST11 CR-HvKP isolates is far higher than that of ST23 and ST65 CR-HvKP (22%, 9 of 41) (Table 1). Although the number of currently identified CR-HvKP isolates is too small (41 isolates), these investigations may imply that the transmission of pLVPK-like plasmids from HvKP strains to CRKP strains seems to be a prevalent event. If the transfer rates of pLVPK-like and blaKPC-2-horboring plasmids between HvKP and CRKP are compared through conjugation experiments, it can provide additional information about plasmid-mediated convergence of carbapenem-resistance and hypervirulence.

Among 78 capsular serotypes of *K. pneumoniae*, K1 and K2 serotypes have known to be strongly associated with HvKP [6,10,25]. However, only 36% (15 of 41) of CR-HvKP isolates belonged to the K1/K2 capsule type (Table 1). Danxia et al. showed that ST11 CRKP strains with the K47 capsular serotype exhibit the hypervirulent phenotype only by acquiring the pLVPK-like plasmid [12]. Therefore, these results imply that more extensive studies are required to exactly establish the relationship between hypervirulence and the K1/K2 capsular type.

Table 1. *Comparison of sequence types and genotypes of bla genes of 36 CR-HvKP strains isolated in China between 2010 and 2018 and 5 CR-HvKP strains from Danxia et al.'s study.*

Features of 36 CR-HvKP strains isolated in China between 2010 and 2018			Features of 5 CR-HvKP strains from Danxia et al.'s study		
Sequence type	**Genotype of *bla* gene**	**Capsule type**	**References**	**Sequence type**	**Genotype of *bla* gene**	**Capsule type**	**Reference**
ST11 (18 isolates)	bla_{KPC-2} (34 isolates)	K1 (7 isolates)	[13-18]	ST11 (5 isolates)	bla_{KPC-2} (5 isolates)	K47 (5 isolates)	[12]
ST65 (6 isolates)	Unknown (2 isolates)	K2 (8 isolates)					
ST23 (3 isolates)		K20 (6 isolates)					
ST1797 (3 isolates)		Non-typeable (15 isolates)					
ST268 (2 isolates)							
ST25 (1 isolates)							
ST85 (1 isolates)							
ST595 (1 isolates)							
ST692 (1 isolates)							

Scope for Further Studies

First, 25% of CR-HvKP strains belonged to ST65 or ST23. Because ST23 and ST65 is the dominant clone of HvKP in China [6], these results imply the transmission of the resistance plasmid from CRKP to HvKP. China is the region with the high prevalence of HvKP as well as CRKP strains [3,6,26].

Therefore, there is always the possibility for an alarming evolutionary event (the plasmid-mediated convergence of carbapenem-resistance and hypervirulence). Because one of two possible mechanisms of the plasmid-mediated convergence was proved, another mechanism (the horizontal transfer of resistance plasmids) of the convergence should be examined. If the horizontal transfer of resistance plasmids into HvKP strains is proved, the potential threat of CR-HvKP strains will increase. Because various features (size, conjugation efficiency, etc.) of the resistance plasmid are different to those of the virulence plasmid, such as the pLVPK-like plasmid, the study about the horizontal transfer of resistance plasmids into HvKP strains may also have a clinical importance.

Second, the horizontal transfer of resistance or virulence plasmids between CRKP and HvKP is not confirmed by conjugation experiment. Although the horizontal transfer of resistance plasmids harboring the bla_{KPC-2} gene to *E. coli* [12] or ST65 HvKP [24] was confirmed through conjugation experiments, it did not examine whether the pLVPK-like plasmid of HvKP can be transferred to CRKP through conjugation. There is only one plasmid sequence (AY378100) of pLVPK or pLVPK-like plasmid deposited in GenBank. The bioinformatic analysis of this sequence shows that any *tra* gene (*traABCDEFGHIKLNPQSTUVWX*) is not present in the pLVPK plasmid. However, the *tra* operon can be located in various intracellular places, including another plasmid in the same host cell and the bacterial genome [27]. In addition, besides conjugation, there are other mechanisms of horizontal gene transfer, such as membrane vesicle and autolysis [28]. Based on these characteristics of the pLVPK or pLVPK-like plasmid, the horizontal transfer of these plasmids from HvKP to CRKP has to be confirmed by conjugation experiment.

Conflict of Interests

The authors declare that they have no competing interests.

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