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Rapid increase in occurrence of carbapenem-resistant Enterobacteriaceae in healthy rural residents in Shandong province, China, from 2015 to 2017

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Highlights

- Feces of residents in rural Shandong, China, were screened for CRE
- An increase in CRE carriage from 2.4% to 13% was observed from 2015 to 2017
- Most CRE isolates (93%) were *Escherichia coli*
- All CRE isolates carried NDM-type carbapenemase genes

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Abstract

Objectives

The global increase of carbapenem-resistant Enterobacteriaceae (CRE) is a growing concern. Infections caused by CRE are associated with increased mortality and length of hospital stay, emphasizing the health and economic burden posed by these pathogens. Although CRE can inhabit the human gut asymptotically, colonization with CRE is associated with increased risk of CRE infection and mortality. In this study, we investigated the occurrence and characteristics of CRE in fecal samples from healthy persons in 12 villages in Shandong, China.

Methods

Screening for CRE in fecal samples was performed by selective cultivation. MICs of meropenem were determined with the agar dilution method. Multilocus sequence type (MLST) and carbapenemase gene carriage of the isolates were determined with whole-genome sequencing. Genetic relatedness of *E. coli* isolates was determined by core genome MLST.

Results

CRE carriage increased from 2.4% to 13% from 2015 to 2017. Most CRE isolates (93%) were *E. coli* and all carried NDM-type carbapenemases. The STs among the *E. coli* were diverse. The single most common was the highly epidemic strain ST167, which was only observed among isolates from 2017.

Conclusion

We report a rapid increase in occurrence of CRE from 2.4% to 13% among fecal samples collected from healthy rural residents of Shandong province, China, from 2015 to 2017.

Colonization with CRE is known to increase the risk of CRE infection and the worrying deterioration of the epidemiological situation in the region reported here indicate a need for further monitoring and possible interventions.

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1. Introduction

Carbapenem-resistant Enterobacteriaceae (CRE) are becoming increasingly common worldwide due to the expansion of high-risk clones and plasmids carrying carbapenemase genes [1-3]. The rapid increase of CRE globally during the last decade has led the US Center for Disease Control and Prevention, the European Centre for Disease Prevention and Control and the World Health Organization to list CRE as prioritized pathogens regarding surveillance, prevention and control in health care [4-6]. Infections caused by CRE are associated with increased mortality and length of hospital stay, emphasizing the health and economic burden posed by these pathogens [7,8]. Although CRE can inhabit the human gut asymptotically, colonization with CRE is associated with increased risk of CRE infection and mortality [9,10]. Monitoring the carriage in healthy individuals is therefore essential for assessing the epidemiological situation regarding CRE.

2. Materials and Methods

In July 2015, we investigated the carriage rates of CRE in a rural region of Shandong province, China, by collecting fecal samples from 758 healthy individual from different households in 12 villages [11]. To investigate the development of the epidemiological situation, the same individuals from 628 of the households were sampled again in July 2017. Additionally, clinical samples were collected continuously from July 2015 until July 2017 from a hospital serving the sampling area. Fecal samples were screened for CRE by cultivation on ChromID CARBA and ChromID OXA-48 agar (bioMérieux, Marcy l'Etoile, France). Suspected CRE-isolates were verified as Enterobacteriaceae by using MALDI-TOF mass spectrometry on a Shimadzu (Shimadzu Corporation, Kyoto,

Japan). Susceptibility to meropenem was determined by using agar dilution and isolates were determined as CRE if the MIC of meropenem >0.125 mg/L in accordance with the EUCAST recommended screening cut-off, and subsequently whole-genome sequenced by Sangon (Shanghai, China) on a HiSeq X Ten (Illumina, San Diego, CA, USA).

Clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* were screened with disc diffusion and confirmed as meropenem-resistant with MIC-determination by using the CLSI clinical breakpoint (≥ 4 mg/L) and subsequently whole-genome sequenced by Novogene Technology (Tianjin, China) on a NovaSeq 6000 System (Illumina).

Relatedness of *E. coli* isolates was determined by using the multi-locus sequence typing (MLST) at enterobase.warwick.ac.uk and core genome MLST (cgMLST) by using the SeqSphere + software (Ridom, Münster, Germany). A detailed study protocol can be found in Sun et al., 2018 [12].

3. Results

3.1 Occurrence of CRE and carbapenemase genes

18 and 84 individuals were positive for CRE in 2015 and 2017 respectively. This corresponds to an increase of CRE occurrence from 2.4% in 2015 to 13% in 2017 (Table 1). 24 individuals positive for CRE in 2017 carried more than one strain, with 20 carrying two strains of *E. coli* (data not shown). Most isolates were *E. coli* ($>90\%$ at both sampling occasions), with one isolate of *Raoultella ornithinolytica* found in 2015, and six isolates of *Enterobacter* spp. and two isolates of *K. pneumoniae* found in 2017. All isolates carried *bla*_{NDM}-genes. The most prevalent carbapenemase gene was *bla*_{NDM-5} (67% and 76% in 2015 and 2017, respectively). An increase in CRE occurrence was observed

in every village except one (Fig. S1). Four CRE were recovered from clinical samples from the hospital (two from urine, one from sputum and one from wound secretion), all of which were *E. coli* carrying *bla*_{NDM-5} (n=3) or *bla*_{NDM-1} (n=1).

3.2 Multilocus sequence type (MLST) diversity of *E. coli* isolates

The *E. coli* sequence types (STs) were diverse, with a total of 53 different STs identified and only 5 STs being represented among isolates from both years (Table 2). The most common were ST167 (n=13) and ST48 (n=12), however, most STs detected were represented by only a single isolate (n=36). The cgMLST also showed a high diversity among STs (Fig. 1A-B, Fig. S2, Fig. S3). For example, isolates belonging to ST167 and ST48 differed in up to 584 and 946 alleles, respectively. However, clonal dissemination was identified between individuals and villages, including isolates belonging to ST5048, ST8666, ST8667, and a subset of ST167, ST48 and ST206 isolates. Among the clinical isolates, ST167 (n=2), ST155 (n=1) and ST410 (n=1) were identified. The two ST167 from the hospital differed only in one cgMLST allele and differed in only 28 alleles to isolates from one village (Fig. 1A-B). No clear trends regarding the distribution of STs and cgMLST between the different villages could be observed (Fig. 1A).

4. Discussion

This study shows a rapid five-fold increase in occurrence of CRE among rural residents in a region of Shandong province, China, from 2015 to 2017. The high increase in carriage of CRE with *bla*_{NDM}-genes in healthy individuals indicates an increased risk of difficult-to-treat infections and the potential for further dissemination of carbapenemase

genes. *E. coli* were the predominant CRE, most of which carried *bla*_{NDM-5}. Based on the results of MLST and cgMLST, the increase of carbapenem-resistant *E. coli* (CREC) was not due to expansion of clonal lineages, indicating that CREC in the villages have either emerged from multiple sources or been introduced at several occasions, or both. Another study investigating fecal carriage of CREC among 3,859 healthy volunteers in 19 provinces in China in 2016 [13] likewise showed *bla*_{NDM-5} to be the most common carbapenemase and was carried by 93% of CREC. That study showed an CREC occurrence of 2.4%, which was similar to that observed in 2015 in the present study (2.2%). Nationwide surveillance studies on CRE in China have identified *K. pneumoniae* producing KPC-2 as the most prevalent CRE causing infections in the country, followed by *E. coli* producing NDM-type carbapenemases. Among *K. pneumoniae*, ST11 predominate whereas *E. coli* are distributed more evenly among several STs, with the most common being ST131 and ST167 [14,15]. In the present study, ST167 was the most prevalent *E. coli* STs (13% in 2017). Two of four hospital isolates were identified as ST167, which also showed genetic relatedness to isolates from the villages, indicating a transmission of ST167 between the hospital and the villages. Interestingly, isolates of ST167 were not observed among samples collected in 2015, but was identified in backyard pigs in the same villages in 2015 [11].

5. Conclusions

The concurrence of a rapid increase in CRE occurrence among rural residents of the study area from 2015 to 2017 with the emergence of the highly epidemic ST167 strain in 2017 and among clinical samples from the regional hospital indicate a worrying

worsening of the epidemiological situation. Further studies and monitoring are essential to understand the changes in occurrence of CRE reported in this study and the potential consequences for public health. Interventions also need to be considered in China to prevent further dissemination of CRE.

Declarations

Ethics approval and consent to participate

All study participants provided informed consent for participating in the study. Ethical approval for this study was granted by the Research Committee of the First Affiliated Hospital, College of Medicine, Zhejiang University, China (2015#185 and 2015#283).

Availability of data and materials

Whole-genome sequence raw reads were submitted to the Sequence Read Archive at the National Center for Biotechnology Information (Accession numbers: SRR11514614-SRR11514723).

Competing interests

The authors declare no conflicts of interest.

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Authors' contributions

BC, SB, MN, HY, YX, ZB and ZB conceived of and designed the study. BC and BZ collected the samples. BC, SW and BZ performed the laboratory analyses. BB, BC, SB, MN, ZB, and LEN analyzed the data. BB wrote the first draft of the manuscript. SB, LEN, BC and MN critically revised the manuscript. All authors approved of the final version of the manuscript.

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Table 1. Information on isolates of carbapenem-resistant Enterobacteriaceae (CRE) originating from fecal samples of healthy individuals in a rural region of Shandong province, China, collected in 2015 and 2017. Fecal samples collected in 2017 were collected from individuals in the same household as fecal samples collected in 2015.

	2015	2017
Sampled individuals	758	628
CRE+ individuals	18 (2.4%)	84 (13%)
CRE-isolates	18	110
Species		
<i>Escherichia coli</i>	17 (94%)	102 (93%)
<i>Klebsiella pneumoniae</i>	0	2 (1.8%)
<i>Enterobacter</i> spp.	0	6 (5.5%)
<i>Raoultella ornithinolytica</i>	1 (5.6%)	0
Carbapenemase gene		
<i>bla</i> _{NDM-1}	4 (22%)	15 (14%)
<i>bla</i> _{NDM-4}	0	2 (1.8%)
<i>bla</i> _{NDM-5}	12 (67%)	84 (76%)
<i>bla</i> _{NDM-7}	0	1 (0.91%)
<i>bla</i> _{NDM-9}	2 (11%)	5 (4.5%)
<i>bla</i> _{NDM-13}	0	3 (2.7%)

CRE=carbapenem-resistant Enterobacteriaceae

Table 2. Sequence types (STs) and carbapenemase genes identified among isolates of carbapenem-resistant *E. coli* from fecal samples collected from healthy individuals in a rural area of Shandong province, China. Only STs represented by three or more isolates are presented separately in the table, isolates belonging to unique STs are categorized as “Others”, whereas isolates for which STs could not be determined are categorized as “Unknown”. Zeros are omitted for clarity.

ST	2015						2017							
	Total	NDM-1	NDM-4	NDM-5	NDM-7	NDM-9	NDM-13	Total	NDM-1	NDM-4	NDM-5	NDM-7	NDM-9	NDM-13
ST10	1 (5.9%)			1 (5.9%)				3 (2.9%)	1 (0.98%)		2 (2.0%)			
ST46	1 (5.9%)	1 (5.9%)						2 (2.0%)			2 (2.0%)			
ST48	3 (18%)			3 (18%)				9 (8.8%)	1 (0.98%)		8 (7.8%)			
ST167								13 (13%)			13 (13%)			
ST206								5 (4.9%)			5 (4.9%)			
ST617								5 (4.9%)	1 (0.98%)		4 (3.9%)			
ST746	1 (5.9%)			1 (5.9%)				6 (5.9%)			6 (5.9%)			
ST6396								3 (2.9%)			3 (2.9%)			
ST8666								7 (6.9%)			7 (6.9%)			
ST8667								3 (2.9%)			3 (2.9%)			
Others ^a	11 (65%)	2 (12%)		7 (41%)		2 (12%)		39 (38%)	2 (2.0%)	2 (2.0%)	27 (26%)	1 (0.98%)	4 (3.9%)	3 (2.9%)
Unknown								7 (6.9%)	5 (4.9%)		1 (0.98%)		1 (0.98%)	

^a This category included the following STs: ST34, ST155, ST156, ST165, ST180, ST181, ST189, ST195, ST218, ST224, ST226, ST297, ST359, ST361, ST410, ST648, ST656, ST711, ST744, ST770, ST1695, ST2854, ST3274, ST1011, ST1114, ST3856, ST3896, ST4429, ST4537, ST4542, ST5044, ST6335, ST6395, ST6449, ST6727, ST6793, ST6910, ST7331, ST7366, ST8153, ST8668, ST8670 and ST10170.

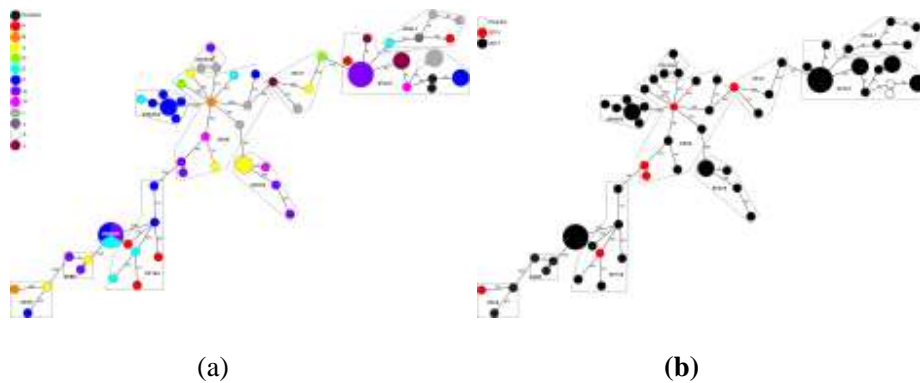


Fig. 1. Minimum spanning tree (MST) of core genome MLST (cgMLST) relationship of carbapenem-resistant *Escherichia coli* carrying bla_{NDM} -genes isolated from fecal samples stratified on the villages from which the sample originated (A) and year the sample was collected (B). Colors denote whether the isolates originated from samples collected at villages (A-L) or the study hospital and which year the sample was collected (2015 or 2017). The cgMLST was defined by using the Enterobase cgMLST database available through the SeqSphere + Software, the ST-numbers denote the ST as defined by the scheme at <https://enterobase.warwick.ac.uk/>, and the brackets mark the nodes belonging to the same ST. The MST includes only 7-MLSTs represented by more than two isolates and is based on 1997 alleles present in all isolates included in the figure. Numbers on the lines between nodes (isolates) indicate differences in alleles (genes) between isolates and the lengths of lines are based on the number of gene differences in a logarithmic scale, while the size of the nodes is proportional to the number of isolates.