Prevalence and molecular characterization of carbapenem-resistant *E. coli* and *K. pneumoniae* at a major university hospital in Thailand

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ABSTRACT

The situation of antimicrobial resistance is dramatically increased worldwide. World Health Organization (WHO) revealed the list of antimicrobial-resistant "critical-priority pathogens" that pose the biggest threat. Carbapenem-resistant *E. coli* (CR-EC) and carbapenem-resistant *K. pneumoniae* (CR-KP) are classified within this group. Accordingly, knowledge of prevalence and molecular characterization of these pathogens are necessary. The prevalence and characterization of CR-EC and CR-KP in Thailand are scarce. The aim of this study was to elucidate the prevalence and molecular characteristic of CR-EC and CR-KP at Siriraj hospital, the largest university medical center in Thailand. The isolates were obtained during October 2017 to July 2018. CRE are defined as *Enterobacteriaeceae* that are resistant to imipenem, meropenem, doripenem or ertapenem, either one or more, or produce carbapenemase(s) according to the CDC guideline. CR-EC and CR-KP were randomly detected for bla_{NDM}, bla_{KPC} and bla_{OXA-48} by using multiplex PCR. Of the 3,725 *E. coli* isolates and 1,793 *K. pneumoniae,* the prevalence of CR-EC and CR-KP were 1.61% (60/3725) and 22.42 %(402/1793), respectively. Antimicrobial profile of both CR-EC and CR-KP were relatively similar. Both of them were resistant to most beta-lactams, but still susceptible to aminoglycosides. CR-EC and CR-KP, respectively, were differently susceptible to netilmicin (81.58% vs. 26.24%), amikacin (73.58% vs. 31.58%), and gentamicin (39.62% vs. 80.26%). Of the 47 CR-EC studied, the bla_{NDM} was the most predominant gene (63.83%), followed by bla_{OXA-48} (21.27%), and 10.64% of them carried both bla_{NDM} and bla_{OXA-48}. Among 138 CR-KP, a majority also harbored bla_{NDM} (67.39%), followed by bla_{OXA-48} (65.22%), and 41.30% of them carried both bla_{NDM} and bla_{OXA-48}. However, bla_{KPC} was not detected in these two organisms. This study has raised a significant concern for a rise of carbapenem resistance problem, especially for CR-KP, and a widespread of the most prominent bla_{NDM} gene.

INTRODUCTION

The occurrence of carbapenem-resistant *Enterobacteriaeceae* (CRE) has spread throughout the world. The common carbapenemase genes that mainly found in *Enterobacteriaceae* are bla_{KPC}, bla_{NDM} and bla_{OXA-48} which belong to the class A, class B and class D carbapenemase, respectively (1). There are several factors that contribute to the dissemination of these carbapenemase genes including international travel, the contamination of resistance genes in the environment or in livestock, selective pressure and gene mobilization on mobile genetic elements. Therefore, understanding of the prevalent rate and characterization of carbapenemase genes in a particular area is important to control genes dissemination. The aim of this study was to elucidate the prevalence and molecular characteristic of CR-EC and CR-KP at Siriraj hospital, the largest university medical center in Thailand.

MATERIALS AND METHODS

Siriraj hospital is the largest hospital in Thailand with a capacity of more than 2,000 beds. There are more than 2,800,000 outpatient visits and 80,000 inpatient admission each year and these patients come from every region of Thailand. Carbapenem-resistant *E. coli* (CR-EC) and carbapenem-resistant *K. pneumoniae* (CR-KP) were obtained from clinical samples isolated from blood, respiratory tract, sterile sites and urinary tract during October 2017 to September 2018. Bacterial identification was obtained by biochemical test and disk diffusion assay had been performed for antimicrobial susceptibility testing. CR-EC and CR-KP were randomly detected for bla_{NDM}, bla_{KPC} and bla_{OXA-48} by using multiplex PCR that validated in this study.

RESULTS

Of the 3,725 *E. coli* isolates and 1,793 *K. pneumoniae,* the prevalence of CR-EC and CR-KP were 1.61% (60/3,725) and 22.42 % (402/1,793), respectively. Most CR-EC and CR-KP were obtained from in-patient (IPD). They were resistant to almost all antimicrobial agents except for aminoglycosides. However, CR-EC and CR-KP, respectively, were differently susceptible to netilmicin (81.58% vs. 26.24%), amikacin (73.58% vs. 31.58%), and gentamicin (39.62% vs. 80.26%) as showed in Figure 1 and Figure 2. Carbapenemase gene detection showed that bla_{NDM} plays an important role in resistance to carbapenem in both CR-EC (30/47, 63.83%) and CR-KP (93/138, 67.39%) followed by bla_{OXA-48} in which 21.27% and 65.22%, respectively. The CR-EC and CR-KP that carry both bla_{NDM} and bla_{OXA-48} were 10.64% (5/47) and 41.30% (57/138) as showed in Figure 4. No bla_{KPC} had been detected in this study.

Carbapenem-resistant *E. coli*

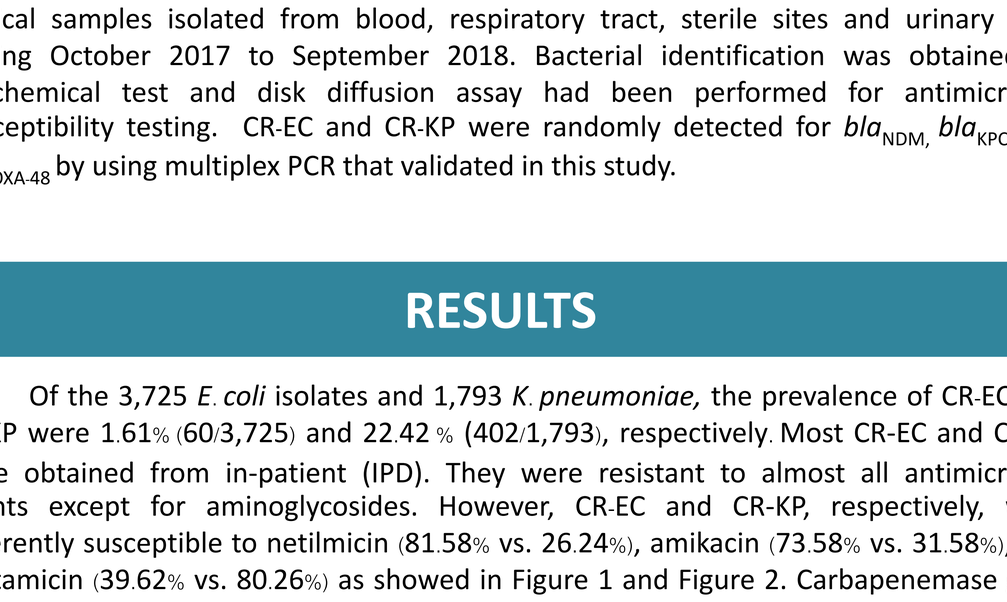

Figure 1. Percent susceptibility of Carbapenem-resistant *E. coli* (CR-EC)

Carbapenem-resistant *K. pneumoniae*

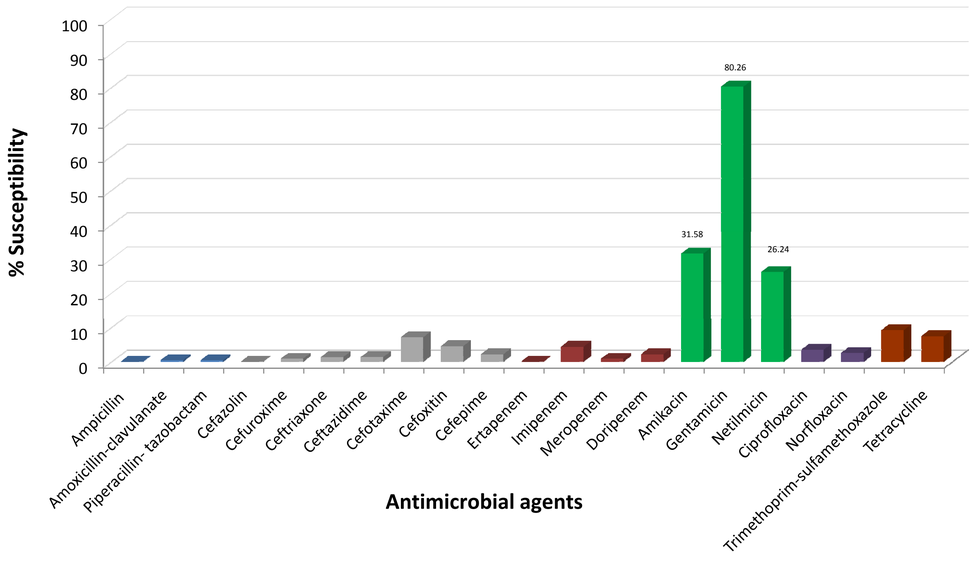

Figure 2. Percent susceptibility of Carbapenem-resistant *K. pneumoniae* (CR-KP)

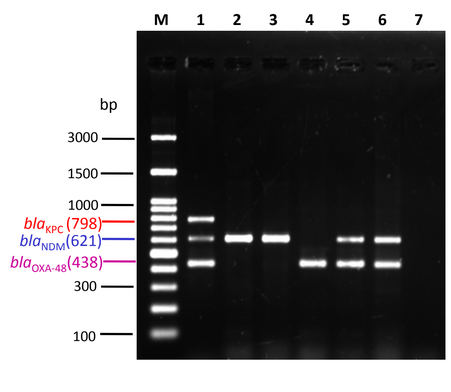

Figure 3. Agarose gel (1.5%) electrophoresis of amplification products of bla_{KPC} bla_{NDM} and bla_{OXA-48} genes obtained from the multiplex PCR.

Lane M: DNA size marker (100-bp ladder)
Lane 1: positive control for of bla_{KPC} bla_{NDM} and bla_{OXA-48}
Lane 3-6:representative clinical CR-EC and CR-KP isolates
Lane 7: negative control (without template)
The length of molecular size markers and amplification products (bp) are indicated on the left.

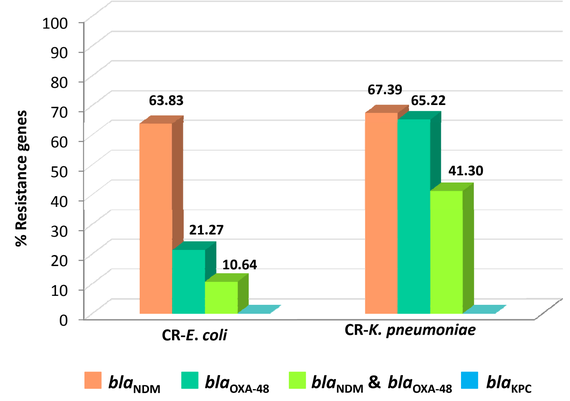

Figure 4. Percentage of bla_{NDM}, bla_{OXA-48}, bla_{KPC} and bla_{NDM} combine with bla_{OXA-48} in CR-EC and CR-KP

DISCUSSION AND CONCLUSION

Carbapenem-resistant *Enterobacteriaceae* becomes a serious threat to public health system. In Thailand, the prevalence of CRE is dramatically increased. Report from Rajvithi hospital manifested that the percentage of CR-KP gradually increased from 1.3% in 2009 to 5.7% in 2012 and 16.4% in 2015, respectively (2). In 2010, the percentage of CR-KP of 4 hospitals in Bangkok and perimeter area was 5.13% (3). Present study, the prevalence of CR-KP was 22.42%. This result showed that the incidence of CR-KP in Thailand increased 4-folds within 8 years. Accordingly, careful monitoring is necessary. Several factors can drive the increase of CRE. Study in China found a significant relationship between carbapenem consumption intensity and the rates of carbapenem-resistant organisms (4). Our study also showed that bla_{NDM} was the dominant genes responsible for carbapenem resistance in both *E. coli* (63.83%) and *K. pneumoniae* (67.39%) followed by bla_{OXA-48}. No bla_{KPC} had been detected in this study. Likewise, the study of 287 CRE isolates in Thailand during 2012 to 2016 indicated that bla_{NDM} and bla_{OXA-48} were predominant genes in which 71.75% and 50.22%, respectively (5). bla_{NDM} spread more rapidly in Thailand, probably because of convenient international travel, the contamination of this resistance gene in environment, community as well as animal production. Besides this, bla_{NDM} can encode different mobile genetic elements that are easily transferable making their rapid dissemination in clinically setting (6). Therefore, implement stringent infection policies are necessary for prevention of their spread.

References

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