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Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction Genotyping of Extended-Spectrum Beta-Lactamase - Producing *Klebsiella pneumoniae* Strains from A Malaysian Teaching Hospital

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ABSTRACT

Klebsiella pneumoniae (KP) is a major cause of hospital-acquired infections (HAIs). The bacteria can acquire plasmids encoding Extended-Spectrum Beta-Lactamases (ESBL) that confer resistance to many antibiotics and lead to treatment failure. This study aimed to determine the prevalence of five ESBL genes (bla_{TEM} + $bla_{CTX\text{-}M\text{-}1}$ + $bla_{CTX\text{-}M\text{-}9}$ + bla_{SHV} + $bla_{OXA\text{-}1}$) and the genetic relatedness of ESBLKP strains isolated from Hospital Canselor Tuanku Muhriz (HCTM), the teaching hospital of Universiti Kebangsaan Malaysia. From December 2023 to May 2024, 31 ESBLKP strains and their antibiotic susceptibility testing (AST) results were obtained from the Department of Diagnostic Laboratory Services. ESBL genotyping was performed using the Polymerase Chain Reaction (PCR), and Enterobacterial Repetitive Intergenic Consensus PCR (ERIC-PCR) was carried out on strains exhibiting the three most common AST profiles. Tested strains were resistant to 3rd -generation cephalosporins, with notable resistance to cefepime (90.3%), amoxicillin-clavulanate (83.9%), ampicillin-sulbactam (67.7%), and ciprofloxacin (51.6%). Worryingly, three strains were carbapenem-resistant. The most prevalent ESBL genes were bla_{SHV} (100%) and $bla_{CTX\text{-}M\text{-}1}$ (90.3%), while 54.8% of the strains carried the combination bla_{TEM} + $bla_{CTX\text{-}M\text{-}1}$ + bla_{SHV} . ERIC-PCR revealed four main clusters with 70% similarity. Our findings demonstrate a high prevalence of bla_{SHV} and $bla_{CTX\text{-}M\text{-}1}$ also highlight the genetic diversity of ESBLKP strains isolated from HCTM patients. In addition to monitoring AST trends, continuous genetic surveillance of ESBLKP will be important.

Keywords: *Klebsiella pneumoniae* (KP); Extended-Spectrum Beta-Lactamase (ESBL); Enterobacterial repetitive intergenic consensus polymerase chain (ERIC-PCR)

1. Introduction

Klebsiella pneumoniae (KP), a gram-negative, oxidase-negative bacterium of the order Enterobacterales, is a member of the gut flora but may cause infections. In recent years, strains of Extended-Spectrum Beta-Lactamase (ESBL)-producing *K. pneumoniae* (ESBLKP) have been isolated, where these strains are commonly resistant to 3rd-generation cephalosporins, extended-spectrum penicillins, and monobactams due to the production of ESBLs such as TEM, SHV and CTX-M. Prior to the year 2000, ESBLKP genotypes were predominantly the TEM and SHV [1]. However, after the

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millennium, CTX-M-type ESBLs were observed to achieve pandemic spread and now constitutes the majority of ESBLKPs globally [2].

Hospital Canselor Tuanku Muhriz (HCTM), the teaching hospital of the University Kebangsaan Malaysia, has also reported the isolation of CTX-M ESBLKP and its rising incidence. This study aimed to determine the prevalence of five ESBL genes (*bla*$_{TEM}$ + *bla*$_{CTX-M-1}$ + *bla*$_{CTX-M-9}$ + *bla*$_{SHV}$ + *bla*$_{OXA-1}$) and to assess the genetic relatedness of ESBLKP strains isolated from HCTM from December 2023 to May 2024.

2. Methodology

2.1 Sample collection

Study strains identified and purified as ESBLKP from patients were collected from the Department of Diagnostic Laboratory Services HCTM, from December 2023 to May 2024.

2.2 Antibiotic susceptibility testing

Results of ESBLKP antibiotic susceptibility testing (AST) using the Vitek 2 system (bioMérieux, France) were obtained from the WHONET system. Antibiotics tested were penicillin (AMP), cefuroxime (CFU), cefotaxime (CTX), ceftriaxone (CRO), ceftazidime (CAZ), cefepime (FEP), amoxicillin-clavulanate (AMC), piperacillin-tazobactam (TZP), ampicillin-sulbactam (SAM), amikacin (AMK), gentamicin (GEN), ciprofloxacin (CIP), imipenem (IMP), meropenem (MEM), and ertapenem (ETP). The AST data were analysed to build antibiotic susceptibility profiles. The three most common AST profiles, along with the associated strains, were identified.

2.3 ESBL genotyping

DNA was extracted from ESBLKP strains using the boiling method [3]. ESBL genotyping was carried out according to the protocol described by Ogutu *et al.* (2015) [4].

2.4 Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR)

ERIC sequences are found in many bacterial genomes, including those of the Enterobacterales, and can be used to assess the genetic similarity of tested strains [5]. DNA extracted for ESBL genotyping was also used for ERIC-PCR typing, as described in Kundu *et al.* (2022) [5].

3. Results & discussions

During our study period, 31 ESBLKP were included in the study. These strains were found to be resistant to several antibiotics (Table 1). The most prevalent ESBL gene detected was *bla*$_{SHV}$, followed by *bla*$_{CTX-M-1}$ (Table 2). AST profile analysis identified six common resistance profiles among the strains (Table 3). ERIC-PCR analysis further demonstrated genetic diversity of the strains, with four main clusters identified at a 70% similarity threshold (Figure 1).

Table 1
AST of ESBLKP isolated from HCTM during the study period

Antibiotic		No. of strains (%)	
		Resistant	Susceptible
Group	**Antibiotic**		
Penicillin	Ampicillin	31 (100)	0 (0)
2nd-generation cephalosporin	Cefuroxime	31 (100)	0 (0)
	Cefotaxime	31 (100)	0 (0)
3rd-generation cephalosporin	Ceftriaxone	31 (100)	0 (0)
	Ceftazidime	31 (100)	0 (0)
4th-generation cephalosporin	Cefepime	28 (90.3)	3 (9.7)
	Amoxicillin–clavulanate	26 (83.9)	5 (16.1)
Beta-lactam/Beta-lactamase inhibitor	Piperacillin–tazobactam	13 (41.9)	18 (58.1)
	Ampicillin–sulbactam	21 (67.7)	10 (32.3)
Aminoglycoside	Amikacin	0 (0)	31 (100)
	Gentamicin	5 (16.1)	26 (83.9)
Fluroquinolone	Ciprofloxacin	16 (51.6)	15 (48.4)
Carbapenem	Imipenem	3 (9.7)	28 (90.3)
	Meropenem	3 (9.7)	28 (90.3)
	Ertapenem	3 (9.7)	28 (90.3)

Table 2
ESBL genotypes of tested strains

ESBL gene	No. of strains (%)
bla_{TEM}	21 (67.7)
$bla_{CTX\text{-}M\text{-}1}$	28 (90.3)
$bla_{CTX\text{-}M\text{-}9}$	0 (0)
bla_{SHV}	31 (100)
$bla_{OXA\text{-}1}$	3 (9.7)
ESBL genotype	
bla_{TEM} + bla_{SHV}	3 (9.7)
$bla_{CTX\text{-}M\text{-}1}$ + bla_{SHV}	8 (25.8)
bla_{TEM} + $bla_{CTX\text{-}M\text{-}1}$ + bla_{SHV}	17 (54.8)
$bla_{CTX\text{-}M\text{-}1}$ + $bla_{OXA\text{-}1}$ + bla_{SHV}	2 (6.4)
bla_{TEM} + $bla_{CTX\text{-}M\text{-}1}$ + $bla_{OXA\text{-}1}$ + bla_{SHV}	1 (3.2)

Table 3
Common AST profiles of tested strains

Most common AST profiles	No. of strains (%)
AMP + CFU + CTX + CAZ + CRO + FEP + SAM + AMC + TZP	4 (12.9)
AMP + CFU + CTX + CAZ + CRO + FEP + SAM + AMC + TZP + CIP	4 (12.9)
AMP + CFU + CTX + CAZ + CRO + FEP + SAM + AMC + TZP + CIP + ETP + IMP + MEM	3 (9.7)
AMP + CFU + CTX + CAZ + CRO + FEP + SAM + TZP	3 (9.7)
AMP + CFU + CTX + CAZ + CRO + FEP + SAM + TZP + CIP	3 (9.7)
AMP + CFU + CTX + CAZ + CRO + FEP	3 (9.7)

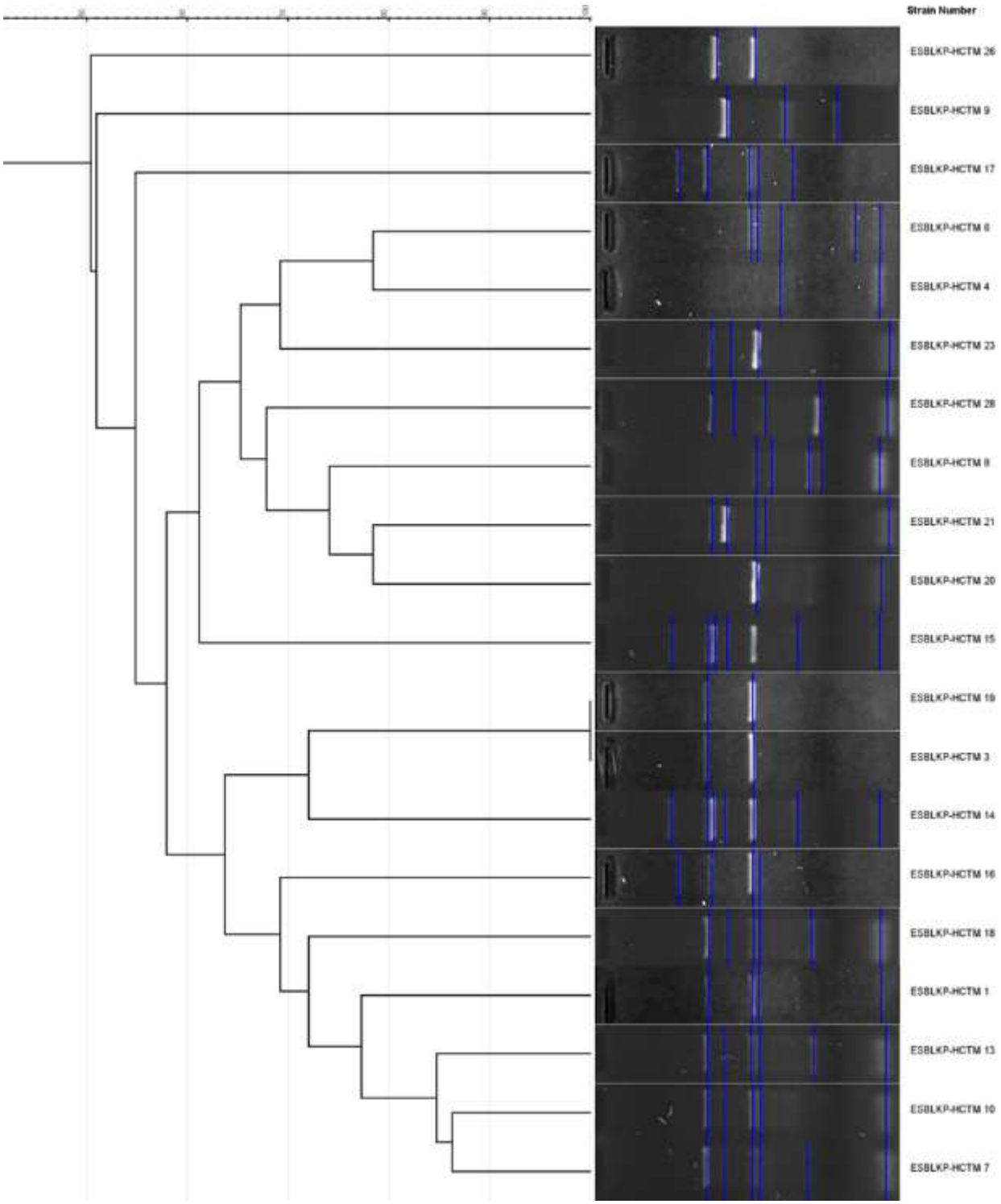

Fig 1. ERIC-PCR analysis of tested strains with the most common AST profiles

Our findings showed that the tested strains were resistant to a broad range of antibiotics, raising concerns about the effectiveness of treatment options. Although the percentage of carbapenem-resistant strains was low (9.7%), this finding suggests the emergence of potential carbapenemase producers, which is of concern as carbapenems are administered as a last resort for severe ESBLKP infections. The predominance of bla_{SHV} and $bla_{CTX\text{-}M\text{-}1}$ highlights their importance in driving resistance among our study strains. bla_{SHV} is commonly associated with *K. pneumoniae* [1], whereas $bla_{CTX\text{-}M\text{-}1}$ has achieved widespread global dissemination [6]. AST profiles and ERIC-PCR analysis revealed possible circulation of diverse clones within HCTM that are resistant to a range of antibiotics.

4. Conclusions

Our findings demonstrate a high prevalence of bla_{SHV} and $bla_{CTX\text{-}M\text{-}1}$, as well as the genetic diversity of ESBLKP strains isolated from HCTM patients. While traditional pathogen surveillance in hospitals is based on AST profiles, analysing these profiles in conjunction with ERIC-PCR typing can enhance the surveillance of circulating strains. Our findings contribute towards ESBLKP surveillance in HCTM.

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