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Brief Communication

Prevalence of extended-spectrum beta-lactamase and carbapenemase-producing bloodstream isolates of *Klebsiella pneumoniae* in a tertiary care hospital

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To improve prescribing of empiric therapy, the local molecular epidemiology of extended-spectrum beta-lactamases (ESBLs) and *Klebsiella pneumoniae* carbapenemases (KPCs) in bloodstream isolates of *K. pneumoniae* were evaluated. Isolates resistant to third generation cephalosporins were screened phenotypically for ESBLs and carbapenemases, and subsequently confirmed by PCR for the presence of ESBL (bla_{TEM}, bla_{SHV} and bla_{CTX-M}) and carbapenemase (bla_{KPC}, bla_{VIM}, bla_{NDM} and bla_{OXA-48}) genes. Hydrolytic activity (functional gene expression) was quantified using a nitrocefin degradation assay and correlated to ceftazidime or meropenem MIC. Clonality was assessed by repetitive element-based PCR. Beta-lactamases were functionally expressed in 13 isolates (15.5%); 7 (53.8%) harboured $bla_{CTX-M-15}$ and 6 (46.2%) carried the bla_{KPC-2} gene. Correlation of hydrolytic activity to MIC yielded a coefficient of 98% for isolates expressing ESBLs alone and 56% for carbapenemase producers. Four unique ESBL-expressing clones and five carbapenem-resistant clones were identified. All 13 resistant isolates were susceptible to ceftazidime/avibactam (MIC ≤ 8/4 mg/L).

Keywords: Gram-negative bacteria, Multidrug resistance, Resistance mechanism, Bacteremia

Introduction

Infections caused by extended-spectrum beta-lactamases (ESBLs) and carbapenemase-producing Enterobacteriaceae account for a significant portion of antibiotic resistance in Gram-negative bacteria and their continued dissemination contributes to poor patient outcomes.[1,2] According to the 2010 data from the SENTRY Antimicrobial Surveillance Program, 175 (89.7%) Enterobacteriaceae bloodstream isolates screened from 26 U.S. hospitals carried at least one beta-lactamase gene.[3] Both the 2010 and 2014 SENTRY data found $bla_{CTX-M-15}$ and bla_{KPC-2} to be most prevalent across all Gram-negative species, with the highest rates encountered in the Mid-Atlantic region of the country.[3,4] In both surveillance reports, *K. pneumoniae* was a key nosocomial pathogen harbouring both ESBL and *Klebsiella pneumoniae* carbapenemases (KPC).

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Although these studies provided an overall epidemiologic representation in a geographic region, the findings were not specific enough to guide local antimicrobial use (e.g. selection of empiric therapy for critically ill patients). As part of the institutional surveillance/antimicrobial stewardship efforts, we investigated the local prevalence of these beta-lactamase(s), their clonal relatedness and the contribution of enzyme activity to antimicrobial susceptibility in *K. pneumoniae* bloodstream isolates from our institution.

Materials and methods

Identification and Selection of Isolates

Bloodstream isolates of *K. pneumoniae* from December 2014 through December 2015 were identified from the Microbiology Laboratory of CHI Baylor St. Luke's Medical Center in Houston, Texas. The isolates were stored at −80 °C in Protect® (Key Scientific Products, Round Rock, TX) storage vials and sub-cultured twice on 5% blood agar plates (Hardy Diagnostics, Santa Maria, CA) for 24 h at 35° prior to use. Susceptibility data

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obtained by the VITEK 2 automated system (BioMérieux Inc., Durham, NC) was retrieved from electronic medical records. Each patient was included once unless he/she had an isolate obtained more than 2 weeks after the initial culture with different susceptibility to at least three classes of antibiotics. Isolates resistant to any third-generation cephalosporin (e.g. ceftazidime) and/or carbapenem (e.g. meropenem) based on Clinical and Laboratory Standards Institute (CLSI) breakpoints[5] were then screened for the presence of ESBLs and carbapenemases. Ethical approval was obtained from the Institutional Review Boards at the University of Houston and CHI Baylor St. Luke's Medical Center. Informed consent was not required as the isolates were obtained as part of standard patient care and the study was retrospective in nature.

Phenotypic screening for enzyme activity

ESBL production was screened by the double-disc synergy test and confirmed by ceftazidime/ceftazidime + clavulanic acid Etest (BioMérieux, Marcy l'Etoile, France).[6] The screening for carbapenemases was performed by Modified Hodge Test (MHT) and combined-disc test using meropenem plus boronic acid (M-PBA) or EDTA.[7] Confirmation for carbapenemase production was done by imipenem/imipenem + EDTA (AB Biodistribution, Solna, Sweden) and ceftazidime/ceftazidime + avibactam (BioMérieux, Marcy l'Etoile, France) Etests.

Detection of ESBL and carbapenemase-encoding genes

Characterization of genes encoding ESBLs (bla_{TEM}, bla_{SHV} and bla_{CTX-M}) and carbapenemases (bla_{KPC}, bla_{VIM}, bla_{NDM} and bla_{OXA-48}) was carried out by PCR and sequencing with primers/conditions previously described.[8–12]

Evaluation of hydrolytic activity

Enzymatic activity of crude cell lysate was initially quantified for isolates harbouring ESBL or carbapenemase genes using a spectrophotometric assay of nitrocefin degradation.[13] In selected isolates, enzymatic activity attributable to ESBL production was further ascertained by liquid chromatography tandem mass spectrometry (LC-MS/MS) quantification of ceftazidime hydrolysis. Similarly, hydrolytic activity mediated by carbapenemase production was confirmed by spectrophotometric detection of imipenem degradation. *K. pneumoniae* ATCC 13883 was used as a negative control, while two well-characterized clinical strains harbouring CTX-M-15 and KPC-2 served as positive controls.[14,15] Enzymatic activity was normalized based on total protein content (Pierce BCA Protein Assay, Rockford, IL) and compared to the reference clinical strains. Finally, hydrolytic activity was correlated to the respective ceftazidime/meropenem MIC using linear regression.

Clonality assessment

Clonal relatedness of selected isolates was evaluated by repetitive element-based PCR.[16] DNA fragments were separated and analysed using the model 2100 bioanalyzer (Agilent Technologies Inc., Santa Clara, CA). Fingerprint patterns were compared using Diversilab software with a Pearson correlation coefficient (Bacterial Barcodes Inc., Athens, GA). Relatedness was defined by a similarity value of >95%.

Results

Of the 84 unique isolates identified from 81 patients, 13 isolates (15.5%) from different patients screened positive for ESBL and/or carbapenemase activity; 5 isolates were found to express the ESBL phenotype and 8 expressed non-metallo carbapenemase activity. Seven (53.8%) isolates harboured $bla_{CTX-M-15}$, and bla_{KPC-2} was found in 6 (46.2%) of the isolates. Except for 3 isolates, all KPC-positive isolates harboured at least one other beta-lactamase gene, as detailed in Table 1. The susceptibilities of the isolates to ceftazidime, ceftazidime/avibactam, meropenem and polymyxin B are also provided in Table 1. Beta-lactamase genes were functionally expressed in all 13 isolates. Comparison of overall hydrolytic activity to MIC yielded a

Table 1 Phenotyping, genotyping and susceptibility (MIC) results of resistant isolates

Isolate	Phenotype	Gene(s) detected	Ceftazidime	Ceftazidime/Avibactam	Meropenem	Polymyxin B
1255	ESBL	CTX-M-15, SHV-28	**>32**	0.25/4	≤0.25	1.5
1416	ESBL	CTX-M-15, SHV, OXA-1	**8**	0.25/4	≤0.25	2
1562	ESBL	CTX-M-15, OXA-1	**16**	0.25/4	≤0.25	2
2301	ESBL	CTX-M-15	**>32**	1.5/4	**16**	0.75
2366	ESBL	CTX-M-15	**>32**	0.5/4	≤0.25	1
1510	CR-Kp	KPC-2, CTX-M-15, SHV-28, OXA-1	**>32**	2/4	**16**	0.75
1188	CR-Kp	KPC-2, CTX-M-15, SHV-28	**>32**	1/4	**16**	0.75
1498	CR-Kp	KPC-2, SHV-28	**>32**	1.5/4	**>16**	**32**
2376	CR-Kp	KPC-2, TEM-79	**>32**	2/4	**16**	1.5
1804	CR-Kp	KPC-2	**>32**	1.5/4	**>16**	3
2090	CR-Kp	KPC-2	**>32**	1.5/4	**16**	2
2228	CR-Kp	KPC-19, SHV	**>32**	1.5/4	**16**	0.75
1575	CR-Kp	KPC-19	**>32**	4/4	**16**	**6**

Note: MIC in mg/L; bold fonts depict resistant phenotypes. ESBL: extended-spectrum beta-lactamase; CR-Kp: carbapenem-resistant *K. pneumoniae*.

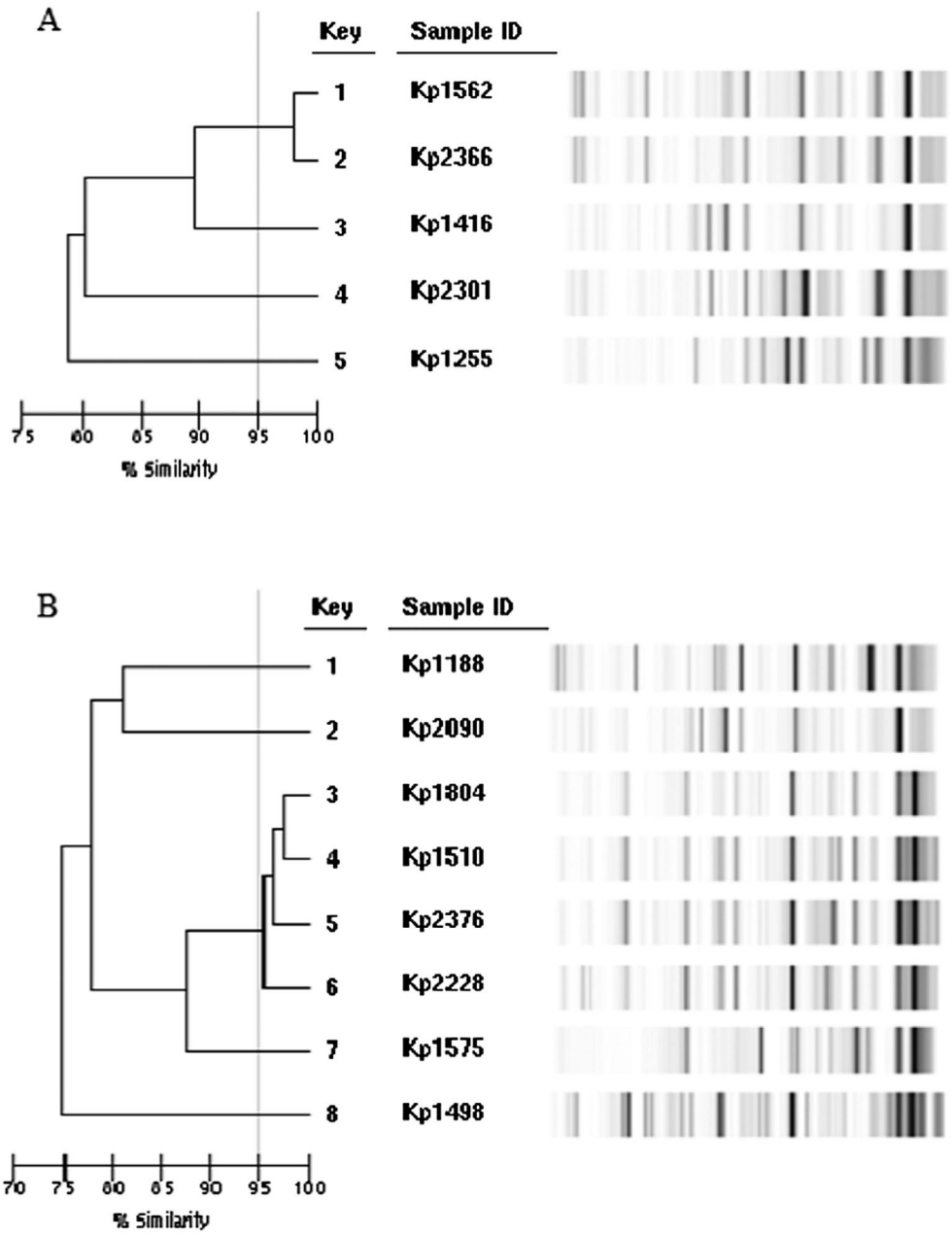

Figure 1 Clonal uniqueness of clinical isolates expressing ESBL (a) and KPC (b).

correlation coefficient of 98% for isolates expressing ESBLs alone and 56% for those expressing carbapenemases (Appendix 1). Additionally, all isolates tested were found to hydrolyze either ceftazidime or imipenem in accordance with their phenotype (Appendix 2). Assessment of clonal relatedness revealed four unique clones in the ESBL-expressing isolates and five unique clones for the carbapenemase-expressing isolates, as shown in Figure 1(a) and (b) respectively.

Discussion

In this study of bloodstream *K. pneumoniae* isolates, the overall prevalence of ESBLs and carbapenemases was lower than that reported nationally.[4] Despite the single centre nature of our study, our isolates were clonally diverse and depicted a predominance of $bla_{\text{CTX-M-15}}$ and $bla_{\text{KPC-2}}$ in agreement with the national profile. In addition to genetic detection of resistance elements, we also used generalized phenotypic and biochemical assays to ascertain that these genes were functionally expressed. Our study demonstrated a trend towards elevated MICs with increased enzymatic activity, especially for isolates with the ESBL phenotype. The correlation was lower in carbapenem-resistant isolates, implying that other non-enzymatic mechanism(s) (e.g. porin deletion or efflux pump over-expression) could be involved.

It is noteworthy that even in KPC-positive isolates with concomitant polymyxin B resistance (i.e. multidrug resistance), the addition of avibactam restored susceptibility to ceftazidime. Results from a recent case study and a randomized trial also substantiated the efficacy of ceftazidime/avibactam, where treatment options were limited.[17,18]

These suggest that beta-lactam/advanced beta-lactamase inhibitor combinations may be a good empiric choice for Enterobacteriaceae when ESBLs or KPCs are suspected. However, prudent use of these agents backed by phenotypic confirmation and optimized dosing regimens are warranted, as clinical resistance to ceftazidime/avibactam with a KPC-3-expressing *K. pneumoniae* isolate has already been reported.[19]

In summary, $bla_{\text{CTX-M-15}}$ and $bla_{\text{KPC-2}}$ were the most prevalent beta-lactamases in bloodstream isolates of *K. pneumoniae* at our institution. We have also established a good correlation between enzymatic activity and ceftazidime susceptibility for ESBL-expressing isolates. Identification of a relationship between phenotypic expression of beta-lactamases and response to beta-lactam/beta-lactamase inhibitor combinations could be instrumental in guiding the appropriate use of empiric antibiotic therapy. Further evaluation of a more diverse population of clinical isolates and risk factors for infection are warranted.

Conflict of interest

No potential conflict of interest was reported by the authors.

Ethical approval

This study was approved by the Institutional Review Boards at the University of Houston and CHI Baylor St. Luke's Medical Center (Houston, Texas, U.S.A.).

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Henrietta Abodakpi is a Pharm.D./Ph.D. candidate at the University of Houston College of Pharmacy working with Vincent Tam. Her research focuses on Gram-negative resistance, particularly resistance mediated by beta-lactamases, and the optimization of dosing for beta-lactam/beta-lactamase inhibitor combinations.

Kai-Tai Chang has a Ph.D. in Chemistry and is currently a research fellow with Tam. His present work focuses on: (1) mechanisms of antimicrobial resistance, (2) pharmacokinetics and pharmacodynamics of antibiotic resistance in bacteria and (3) the clinical impact of antibiotic resistance.

Ana María Sánchez-Diáz is a clinical microbiologist at the University Hospital Ramón y Cajal in Madrid, Spain. Her research is focused on the study of population biology and characterization of multidrug resistant microorganisms, mainly *Enterococcus*.

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At the time of the study, Todd Lasco was the Section Chief of Clinical Microbiology and Molecular Pathology at CHI Baylor St. Luke's Medical Center.

Katrina Chan is a Ph.D. student with Tam. She is currently working on elucidating the mechanism(s) of renal toxicity for selected antibiotics. Her interests include pharmacokinetic modelling of antimicrobials.

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Vincent Tam is a professor in the Department of Pharmacy Practice and Translational Research, and the Department of Pharmacological and Pharmaceutical Sciences at the University of Houston College of Pharmacy. His research interests include pharmacokinetics and pharmacodynamics of antimicrobials, mathematical modelling and simulation of biological processes and the mechanisms underlying bacterial resistance.

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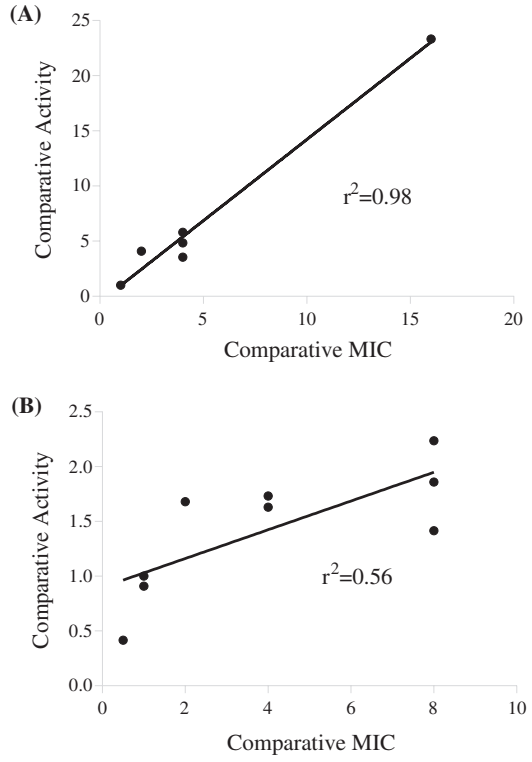
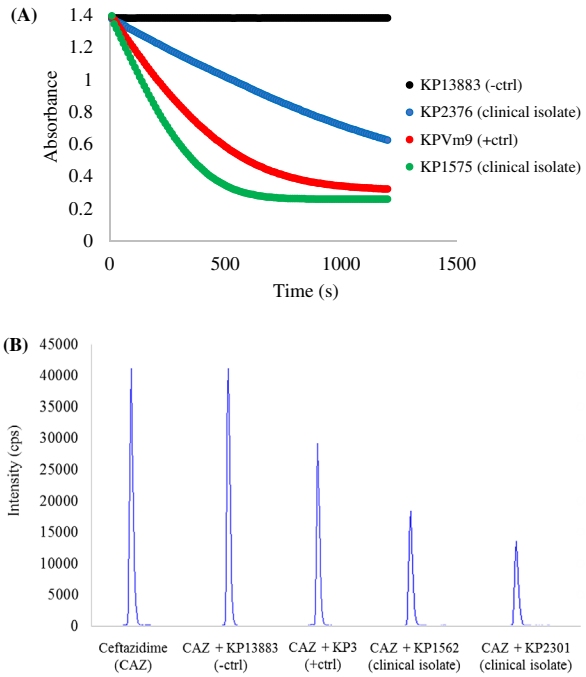
Appendix 1

Correlation of MIC to hydrolytic activity for isolates expressing ESBL (A) and KPC phenotype (B).



Appendix 2

Representative results for imipenem (A) and ceftazidime (B) hydrolysis. Note: Residual ceftazidime concentration was assessed after 60 min of incubation.



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