BRITISH JOURNAL OF BIOMEDICAL SCIENCE
2018, VOL. 75, NO. 4, 200–202
https://doi.org/10.1080/09674845.2018.1492207

BIOMEDICAL SCIENCE IN BRIEF

Molecular characterization of plasmid-encoded *blaTEM, blaSHV* and *blaCTX-M* among extended spectrum *β*-lactamases [ESBLs] producing *Acinetobacter baumannii*

ASG Smiline[a], JP Vijayashree[b] and A Paramasivam[c]

[a]Department of Microbiology, Saveetha Dental College and Hospitals, [SIMATS], Chennai, India; [b]BRULAC-DRC, Saveetha Dental College and Hospitals, [SIMATS], Chennai, India; [c]Centre for cellular and Molecular Biology, Hyderabad, India

ARTICLE HISTORY Received 9 June 2018; Accepted 10 June 2018
KEYWORDS *A.baumannii*; cephalosporins; *blaTEM*; *blaSHV*; *blaCTX-M*

Acinetobacter baumannii associated with nosocomial infections are among the top six drug resistant microbes. Extensive use of the *β*-lactam group of antibiotics has resulted in the emergence of drug resistance and has raised a major clinical crisis. Among the newer *β*-lactamases, extended spectrum *β*-lactamases (ESBLs) have emerged as a major cause of resistance against cephalosporins. A variety of molecular mechanisms such as mutations of penicillin-binding proteins (PBP's), alterations of membrane permeability and chromosomal or plasmid borne cephalosporinases induced by *β*-lactamases (*bla*-genes) viz., *bla*TEM, *bla*SHV and *bla*CTX-M in inducing resistance among *A.baumannii* have been reported [1].

Plasmid-encoded ESBLs are commonly reported to mediate resistance to penicillin, cephalosporins and monobactams, and have been found worldwide among *Enterobacteriaceae, Pseudomonas aeruginosa* and *A.baumannii* species [2]. Detection and molecular characterization of these genes are routinely performed by PCR, specific multiplex PCR assays, and multi-locus sequence typing (MLST), which optimize and facilitate the monitoring of the spread and emergence of ESBL producers of *A.baumannii*. Since these ESBL genes are transmissible and ESBL-related cephalosporin resistance being reported in many parts of the world [3], there is now a developing need to detect the ESBL producers among potentially important and under-researched uropathogens such as *A.baumannii* that are especially encountered in patients with long term hospital stay [4]. Our study is designed to contribute to mapping the ESBL molecular signature of *A.baumannii* by screening for ESBL producers and its genetic relatedness of resistance associated with plasmid-encoded *bla*TEM, *bla*SHV and *bla*CTX-M genes.

From 1000 urine samples of patients with severe urinary tract infections (UTI), we isolated 73 consecutive, non-repetitive *A.baumannii* strains. Estimation of the sample size was done based on the formula n = (Z1-α)2 (P (1 − P)/D2) [5]. The prevalence was set approximately at 50%, with a 95% confidence interval, 5% precision and power set as 80%. Inclusion criteria were the selection of the UTI patients who manifested with specific urinary tract symptoms viz., frequency and urgency of urination with suprapubic discomfort, dysuria, flank pain and without prior administration of antibiotics. All the other strains were excluded from the study. Collection of specimens included random sampling technique regardless of the demographic data. Ethical committee approval and informed consent from all participants were obtained. All the *A.baumannii* strains were phenotypically and genotypically confirmed by conventional microbiological analytical tests and by PCR, respectively. Confirmed strains were further subjected for specific analysis for ESBL producers among *A. baumannii*.

Ceftazidime [30 µg], cefotaxime [30 µg], ceftriaxone [30µg] and aztreonam [30 µg] were used for the initial screening of ESBL producers, and to improve the sensitivity of the ESBL detection, more than one antibiotic agent in a Kirby–Bauer disc diffusion assay was used [6]. Briefly, 0.5 McFarland standards of *A.baumannii* isolates were made as lawn culture on a sterile Mueller Hinton agar plate. The discs were placed on the surface of the plate and the zone diameter was interpreted in comparison with the CLSI criteria, after incubation at 37 °C/24 hrs [6]. Double disc synergy testing (DDST) was performed to detect the ESBL production among the *A.baumannii* isolates by standard Kirby–Bauer disc diffusion method. Briefly, broth suspension of *A.baumannii* isolates was adjusted to 0.5 McFarland standards. Lawn cultures of the broth were made onto sterile Mueller Hinton agar plates. Cephalosporin discs viz., ceftazidime (30 µg), cefotaxime (30 µg) and ceftriaxone (30 µg) [HiMedia Paper discs, Mumbai, India] were placed around the amoxicillin-clavulanate [20/10 µg] discs at a centre-to-

CONTACT ASG Smiline smilinejames25@gmail.com Department of Microbiology, Saveetha Dental College and Hospitals, [SIMATS], Poonamallee High Road, Chennai-600 077, Tamilnadu, India
© 2018 British Journal of Biomedical Science

centre distance of 15 mm from the central disc. An isolate was considered to be an ESBL producer if there was any enhancement of the zone diameter between any of the four cephalosporins against the amoxicillin – clavulanate disc and if there is no enhancement, the strain was considered as ESBL non-producer. ESBL 25922 was used as the negative control and in-house strain of ESBL producing *A. baumannii* was used as the positive control.

ESBL production by *A.baumannii* isolates was done retrospectively on the ESBL producers as confirmed by DDST test by the disc potentiation test [6]. Four antibiotic discs were used for the phenotypic confirmatory test viz., cefotaxime (30 µg), cefotaxime/clavulanic acid (30 µg/10 µg), ceftazidime (30 µg) and ceftazidime/clavulanic acid (30 µg/10 µg). ESBL production by *A.baumannii* isolates was interpreted by observing a >5 mm increase in zone diameter for either antibiotic tested in combination with clavulanic acid versus its zone diameter when tested alone [6].

Fresh cultures were made onto Mac Conkey agar at 37 °C for 24 h prior extraction of plasmid DNA. Extraction of plasmid DNA from *A.baumannii* was done using Qiagen extraction kit in accordance with the manufacturer's instructions. The extracted plasmid DNA was stored at −20 °C until further use. Forward and reverse primers [Eurofins Genomic India Pvt Ltd, Bangalore, India] for *blaTEM* were 5′-ATGATGATTCAACATTTCCG-3′ and 5′-CCAATGCTTAATCAGTGAGG-3′, *blaSHV* were 5′-TTATCTCCCTGTTAGCCACC-3′ and 5′-GATTTGCTGATTTC GCTCGG-3′ and for *blaCTX-M* were 5′- CGCTTTGCGA TGTGCAG-3′ and 5′- ACCGCGATATCGTTGGT −3′, respectively. The selected genes were amplified at appropriate temperatures and sequenced bidirectionally using BigDye terminator cycle sequencing kit and 3730XL Genetic Analyzer. Sequences from forward and reverse primers were aligned using BioEdit Sequence Alignment Editor v7.2.5 which were subjected to BLAST (Basic Local Alignment Search Tool) for nucleotide similarity search.

Preliminary screening of the ESBL producers among the 73 *A.baumannii* isolates found all were resistant to more than one of the antibiotics. DDST found 38.3% (n = 28) and the combination disc method (CDM) found 42.5% (n = 31) of the isolates to be ESBL producers. Molecular characterization of *blaTEM, blaSHV* and *blaCTX-M* showed PCR positivity of 57.5% (n = 42) for *blaTEM* followed by 6.8% (n = 5) for *blaSHV*, but none of the strains showed the presence of *blaCTX-M*. Three (4.1%) isolates showed the presence of both *blaTEM* and *blaSHV*. DDST positive isolates showed 30.1% (n = 22) and 1.4% (n = 1) of *blaTEM* and *blaSHV*, respectively, with the CDM confirmed strains showing 32.9% (n = 24) and 2.7% (n = 2) of the isolates positive for *blaTEM* and *blaSHV*, respectively (Table 1). The amplicons subjected to sequence and BLAST analysis returned results confirming the sequence similarity of *blaTEM* and *blaSHV* genes in various strains of *A.baumannii* (Figure 1 (a,b)).

ESBL encoded by mobile genes in *A.baumannii* among hospitalized patients questions the use of cephalosporins and initiates the use of carbapenems, ultimately resulting in a higher incidence of multidrug resistance [7–14]. The present investigation has recorded 100% resistance to cefotaxime and ceftazidime with 92% resistance to ceftriaxone. Various studies show differences in the incidences of ESBL production among *A.baumannii*, with incidences varying from 28–84% [10]. The present investigation has detected slightly more ESBL producers by CDM when compared to DDST. Larger numbers of ESBL producers were detected using ceftazidime in comparison with cefotaxime. Nearly a fifth of ESBL producers were not detected by cefotaxime when compared to less than 10% of the non-detectable ESBL producers by

Table 1. Preliminary screening and phenotypic confirmation of ESBL producers among 73 *A.baumannii* isolates.

Phenotypic confirmatory tests			
DDST		CDM	
Antibiotics	Positive (%)	Antibiotics	Positive (%)
Ceftazidime (30 µg) Cefotaxime (30 µg) Ceftriaxone (30 µg) around Augmentin (20 µg/10 µg)	38.3	Cefotaxime (30 µg) & Cefotaxime/clavulanic acid (30 µg/10 µg) Ceftazidime (30 µg) & Ceftazidime/clavulanic acid (30 µg/10 µg)	42.5

DDST – Double disc synergy test, CDM – Combination disc method.

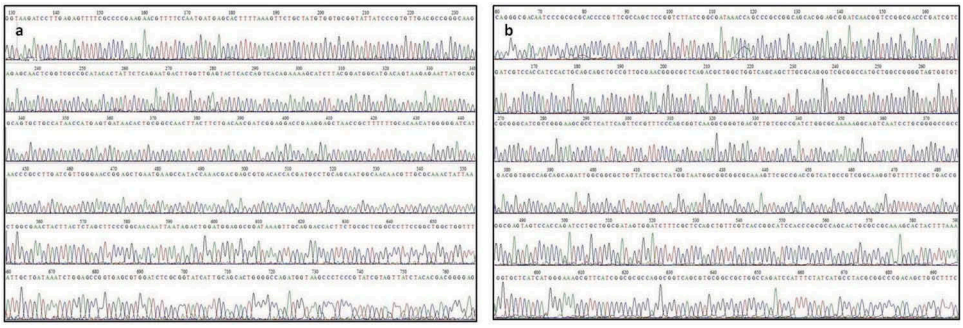

Figure 1. (a) The partial sequence chromatogram of bla_{TEM} gene (b) bla_{SHV} amplified using plasmid DNA as the template isolated from *A.baumannii.*

ceftazidime, suggesting the significance of incorporating ceftazidime in the screening of ESBL producers.

Attempts were made in the present investigation to determine the molecular detection of the resistance phenotype by PCR analysis for *blaTEM, blaSHV* and *blaCTX-M*. Higher rates of the presence of *blaTEM* were reported in many studies with less or no incidence of *blaSHV* and *blaCTX-M*, respectively [11]. We found over half the resistant isolates had *blaTEM*, fewer than a tenth had *blaSHV*, but none had *blaCTX-M*. Elsewhere [12], ESBL producing *A.baumannii* has shown a frequency of 71% of *blaTEM* and 81% of *blaCTX-M* with the absence of *blaSHV*. In another study, low incidences of ESBL genes have been reported despite a high incidence of ESBL producers by phenotypic assay which correlates with the present study. Genotypic detection is sensitive and specific when compared to the phenotypic methods. In the present study, a quarter of ESBL producing *A.baumannii* were confirmed by *blaTEM* detection alone, but only one isolate was confirmed by *blaSHV* detection. All of these isolates were negative for ESBL when observed by phenotypic methods. PCR negative for *blaCTX-M* in the present investigation confirms other data [13].

The presence of multiple ESBL genes is not uncommon [15]. The present investigation has recorded a low frequency of both *blaTEM* and *blaSHV* in the isolates, emphasizing the severity of resistance patterns against cephalosporins. The sequence analysis of the target gene products revealed total identity with various clinical strains of *A.baumannii*, adding to knowledge of ESBL prevalence and epidemiological pattern of resistant strains. However, a comparative analysis on the phenotypic and genotypic data variations is in the present study when compared with the earlier reports. Significance related with the detected genes in comparison with the phenotypic detection of ESBL producers lacks clarity. This suggests the role of alternate genes involved in ESBL production in addition to those we have investigated in the phenotypic and genotypic characterization of ESBL producing *A.baumannii* strains and the subsequent drug resistance that prevails among UTI cases and it requires further investigations. We recommend the periodic antibiotic surveillance for ESBL producers in order to curb the spread of ESBL producing *A.baumannii* strains in hospitalized patients.

This study represents an advance in biomedical science as it demonstrates roles for *blaTEM, blaSHV* and *blaCTX-M* in determining the antibiotic sensitivity or resistance of ESBL producers of *A.baumannii* for different antibiotics in patients with UTI.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

[1] Fernández-Cuenca F, Martínez-Martínez L, Conejo MC, et al. Relationship between β-lactamase production, outer membrane protein and penicillin-binding protein profiles on the activity of carbapenems against clinical isolates of *Acinetobacter baumannii*. J Antimicrob Chemother. 2003;51:565–574.

[2] Rawat D, Nair D. Extended-spectrum β-lactamases in Gram negative bacteria. J Glob Infect Dis. 2010;2 (3):263–265.

[3] Yong D, Shin JH, Kim S, et al. High prevalence of PER-1 extended-spectrum beta-lactamase–producing *Acinetobacter* spp. in Korea. Antimicrob Agents Chemother. 2003;47:1749–1751.

[4] Thomson KS. Extended-Spectrum-Lactamase, AmpC, and Carbapenemase Issues. J Clin Microbiol. 2010;4:1019–1025.

[5] Suresh K, Chandrashekara S. Sample size estimation and power analysis for clinical research studies. J Hum Reprod Sci. 2012;5:7–13.

[6] Clinical Laboratory Standards Institute [CLSI]. 2012. Table 3A:M02-A12 and M07-A10. Available from: https://clsi.org/ Access date: 1st October 2013.

[7] Kahlmeter G. Breakpoints for intravenously used cephalosporins in *Enterobacteriaceae* EUCAST and CLSI breakpoints. Clin Microbiol Infect. 2008;14 (1):169–174.

[8] Kansal R, Pandey A, Asthana AK. Beta-lactamase producing *Acinetobacter* species in hospitalized patients. Indian J Pathol Microbiol. 2009;52:456–457.

[9] Szabo D, Szentandrassy J, Juhasz Z, et al. Imported PER-1 producing *Pseudomonas aeruginosa*. PER-1 producing *Acinetobacter baumannii* and VIM-2-producing *Pseudomonas aeruginosa* strains in Hungary. Ann Clin Microbiol Antimicrob. 2008;7:12.

[10] Bazargani A, Hashemizadeh Z. Bacteremia due to multidrug-resistant (MDR) and extended-spectrum beta-lactamase (ESBL) producing *Acinetobacter baumannii*. Afr J Microbiol Res. 2011;5(21):3483–3486.

[11] Nagano N, Nagano Y, Cordevant C, et al. Nosocomial transmission of CTX-M-2 β-lactamase producing *Acinetobacter baumannii* in a neurosurgery ward. J Clin Microbiol. 2004;42:3978–3984.

[12] Essam Alyamani J, Khiyami MA, Rayan Booq Y, et al. Molecular characterization of extended-spectrum beta-lactamases (ESBLs) produced by clinical isolates of *Acinetobacter baumannii* in Saudi Arabia. Ann Clin Microbiol Antimicrob. 2015;14:38–45.

[13] Marzieh S, Nejad ASM, Bahador A, et al. Prevalence of ESBL and MBL encoding genes in *Acinetobacter baumannii* strains isolated from patients of intensive care units (ICU) Saudi. J Biol Sci. 2015;22 (4):424–429.

[14] Bali NK, Fomda BA, Bashir H, et al. Emergence of carbapenem-resistant Acinetobacter in a temperate north Indian State. Br J Biomed Sci. 2013;70 (4):156–160.

[15] Naas T, Cuzon G, Bogaerts P, et al. Evaluation of a DNA microarray (Check-MDR CT102) for rapid detection of TEM, SHV, and CTX-M extended-spectrum beta-lactamases and of KPC, OXA-48, VIM, IMP, and NDM-1 carbapenemases. J Clin Microbiol. 2011;49 (4):1608–1613.