

## ORIGINAL ARTICLE

# Distribution of *adeB* and *NDM-1* genes in multidrug resistant *Acinetobacter baumannii* isolated from infected wound of patients admitted in a tertiary care hospital in Bangladesh

Mohammad Jahirul HASAN *MBBS, MPhil* and SM SHAMSUZZAMAN *MBBS, PhD*

Department of Microbiology, Dhaka Medical College, Dhaka, Bangladesh

### Abstract

**Background:** The *adeB* gene in *Acinetobacter baumannii* regulates the bacterial internal drug efflux pump that plays a significant role in drug resistance. The aim of our study was to determine the occurrence of *adeB* gene in multidrug resistant and New Delhi metallo-beta-lactamase-1 (NDM-1) gene in imipenem resistant *Acinetobacter baumannii* isolated from wound swab samples in a tertiary care hospital of Bangladesh. **Methods:** A total of 345 wound swab samples were tested for bacterial pathogens. *Acinetobacter baumannii* was identified by culture and biochemical tests. Antimicrobial susceptibility pattern was determined by the disc diffusion method according to CLSI standards. Extended spectrum beta-lactamases were screened using the double disc synergy technique. Gene encoding AdeB efflux pump and NDM-1 were detected by Polymerase Chain Reaction (PCR). **Results:** A total 22 (6.37%) *Acinetobacter baumannii* were identified from 345 wound swab samples and 20 (91%) of them were multidrug resistant. High resistance rates to some antibiotics were seen namely, cefotaxime (95%), amoxycyclavulanic acid (90%) and ceftriaxone (82%). All the identified *Acinetobacter baumannii* were sensitive to colistin and 82% to imipenem. Two (9%) ESBL producing *Acinetobacter baumannii* strains were detected. *adeB* gene was detected in 16 (80%) out of 20 multidrug resistant *Acinetobacter baumannii*. 4 (18%) of 22 *Acinetobacter baumannii* were imipenem resistant. *NDM-1* gene was detected in 2 (50%) of the imipenem resistant strains of *Acinetobacter baumannii*. **Conclusion:** The results of this study provide insight into the role of *adeB* gene as a potential regulator of drug resistance in *Acinetobacter baumannii* in Bangladesh. *NDM-1* gene also contributes in developing such resistance for *Acinetobacter baumannii*.

**Keywords:** *Acinetobacter baumannii*, multidrug resistant, *adeB* gene, New Delhi metallo-beta-lactamase-1

### INTRODUCTION

The emergence and quick dissemination of multiple drug resistant (MDR) *Acinetobacter baumannii* and its genetic potential to carry and transfer diverse antibiotic resistance determinants has become a major threat in hospitals worldwide.<sup>1,2</sup> There are reports of newer broad spectrum  $\beta$ -lactamases such as New Delhi-metallo-beta-lactamase-1 (NDM-1) in *Acinetobacter baumannii*.<sup>3</sup> AdeABC efflux pump mediated antibiotic resistance includes resistance to aminoglycosides,  $\beta$ -lactams, chloramphenicol, erythromycin, tetracyclines, and the dye ethidium bromide in *Acinetobacter*

*baumannii*.<sup>4</sup> This pump is composed of AdeA, AdeB, and AdeC proteins where AdeB is a member of the resistance-nodulation-division (RND) efflux pump superfamily.<sup>5</sup> Ninety-two percent of the multidrug resistant *Acinetobacter baumannii* are positive for *adeB* gene whereas *adeFGH* are positive in 50% of the multidrug resistant isolates.<sup>6</sup>

We undertook this study to explore the incidence of multidrug resistant *Acinetobacter baumannii* in wound infection samples, in a tertiary care hospital of Bangladesh along with the detection of *adeB* gene in multidrug resistant and *NDM-1* gene among the imipenem resistant strains of *Acinetobacter baumannii*.

## MATERIALS AND METHODS

### Ethical clearance

This was a cross sectional study done from January to December, 2013. The research protocol was approved by the research review committee (RRC) and ethical review committee (ERC) of the hospital. 345 wound swab samples were tested. Wound swab samples were collected from hospitalized patients of different wards of Dhaka Medical College Hospital, Dhaka, Bangladesh.

### Identification of *Acinetobacter baumannii*

Wound swab samples collected from patients were inoculated on MacConkey agar media and blood agar media. From the non-lactose fermenting colonies on MacConkey agar media organisms were isolated as *Acinetobacter baumannii* if they were gram-negative coccobacilli, oxidase negative, non-motile, indole and urease negative, citrate positive and grew at 41°C and 44°C.<sup>7</sup> Additional bacterial characteristics including its colony morphology, haemolytic criteria and pigment production were also used to identify the species.

### Antimicrobial susceptibility testing:

Antimicrobial susceptibility test was performed according to CLSI standards using the disk diffusion technique on Muller-Hinton agar media using commercially available antibiotic disks (Oxoid Ltd, Basingstoke, United Kingdom).<sup>8</sup> The isolated organisms were tested against gentamycin (10 µg), amikacin (30 µg), imipenem (10 µg), aztreonam (30 µg), ciprofloxacin (5 µg), cefotaxime (30 µg), ceftazidime (30 µg), piperacillin (100 µg), colistin (10 µg), amoxycylav (20/10 µg), ceftriaxone (30 µg). *Eschericia coli* ATCC 25922 was used for quality control.

### Operational definitions

ESBL: Organisms showing a clear augmentation of the edge of the inhibition zone of cephalosporin (ceftriaxone, ceftazidime and cefotaxime) disk towards amoxicillin-clavulanic acid disk on Muller-Hinton agar media was interpreted as positive for ESBL producing phenotype.<sup>11</sup>

Multi-drug resistant organism (MDRO): An isolate was considered multi-drug resistant if it was resistant to three or more classes of antibiotics among quinolones, cephalosporins, penicillins, carbapenems, polymyxins and aminoglycosides.<sup>10</sup>

Double disk synergy (DDS) test for phenotypic detection of ESBL producers<sup>11</sup>: Using sterile

cotton swab, test inoculums (compared with 0.5% McFarland standard) were inoculated in Muller-Hinton agar plate. Amoxicillin-clavulanic acid disk was placed at the center of the inoculated Mueller-Hinton agar plate and third generation cephalosporins (ceftriaxone, ceftazidime and cefotaxime) were placed 15 mm apart from center of the amoxicillin-clavulanic acid disk. After incubation at 37°C for 24 hours, if there was clear augmentation of any of the cephalosporin inhibition zone towards amoxicillin-clavulanic acid disk, an organism was classified as having an ESBL producing phenotype.

### Molecular characterization of *adeB* and *NDM-1* gene

The presence of *adeB* gene in multidrug resistant isolates and *NDM-1* gene in imipenem resistant isolates of *Acinetobacter baumannii* was detected by polymerase chain reaction (PCR).

### Preparation of bacterial pellets:

To prepare bacterial pellets, a loop full of bacterial colonies was introduced into a falcon tube containing trypticase soy broth. After overnight incubation at 37°C, the tubes were centrifuged at 4,000 g for 10 minutes, after which the supernatant was discarded. A small amount of sterile trypticase soy broth was added into the falcon tubes with pellets and mixed evenly. An equal amount of bacterial suspension was then placed into 2-3 eppendorf tubes. The eppendorf tubes were then centrifuged at 4,000 g for 10 minutes and the supernatant was discarded. The eppendorf tubes containing the bacterial pellets were then kept at -20°C until DNA extraction.

### Extraction of DNA:

Bacterial DNA was extracted by the boiling method.<sup>12</sup> Bacterial pellets were suspended in 300 µl of sterile distilled water in micro-centrifuge tube and were boiled for 10 minutes in heat block. The tube was then placed immediately into ice and kept for 5 minutes. After centrifugation at 14,500 g for 6 minutes, 10 µl of supernatant was used for PCR.

### Amplification of DNA:

The following pairs of previously used primers were used to yield PCR products: for *adeB*—GTA TGA ATT GAT GCT GC (forward), CAC TCG TAG CCA ATA CC (reverse), for *bla<sub>NDM-1</sub>*—GCG CAA CAC AGC CTG ACT TT (forward), CAG CCA CCA AAA GCG ATG TC (reverse).<sup>4,13</sup> The following cycling parameters were used: initial

denaturation at 95°C for 10 minutes, then 30 cycles of denaturation at 95°C for one minute, annealing at 63°C (for *bla<sub>NDM-1</sub>*), 53°C (for *adeB*) for 45 seconds, extension at 72°C for one minute and 30 seconds, and a final extension at 72°C for 10 minutes. *adeB* gene was detected at 979 bp.<sup>4</sup> *NDM-1* gene was detected at 155 bp.<sup>13</sup>

*Visualization of the amplified products:*

The amplified DNA were loaded into a 1.5% agarose gel, electrophoreses at 100 volts for 35 minutes, stained with 1% ethidium bromide, and visualized under UV light.

*Statistical analysis:*

Data were analyzed by using Microsoft Excel (2007) software.

**RESULTS**

Of the 345 samples, 165 (47.72%) were from burn wounds, 80 (23.18%) were surgical wounds, 55 (15.94%) were traumatic wounds and 45 (13.06%) were orthopedic wounds. Among the 345 wound swab samples, 22 (6.37%) yielded growth of *Acinetobacter baumannii*; 14 (8.48%) of them were isolated from burn wound swab. The majority (72%) of *Acinetobacter baumannii* were identified in the age group above 30 years with a male predominance (64%).

Regarding antimicrobial resistance pattern, 20 (90.90%) of the 22 *Acinetobacter baumannii* were found multidrug resistant. Twenty one (95.45%) were resistant to cefotaxime, 20 (90.90%) to amoxycyclavulanic acid and ceftazidime, 18 (81.81%) to ceftriaxone, 16 (72.72%) to ciprofloxacin, aztreonam and gentamicin and 14 (63.63%) to amikacin. All

(100%) were susceptible to colistin, 18 (81.81%) to imipenem and 14 (63.63%) to piperacillin. Four (18.18%) isolates were found resistant to imipenem (Table 1).

Of the isolated 22 *Acinetobacter baumannii*, 2 (9%) were ESBL producers. All (100%) the isolated ESBL producer *Acinetobacter baumannii* showed resistance to amoxycyclavulnic acid and ciprofloxacin but were sensitive to imipenem and colistin. Out of 20 multidrug resistant *Acinetobacter baumannii*, 16 (80%) were positive for *adeB* gene (Fig. 1). None of the non-multidrug resistant *Acinetobacter baumannii* were positive for *adeB* gene. Among the 4 imipenem resistant *Acinetobacter baumannii*, 2 (50%) were positive for *bla<sub>NDM-1</sub>* gene (Fig. 2).

**DISCUSSION**

During the last decades, *Acinetobacter baumannii* has emerged globally as an important nosocomial pathogen that gives rise to outbreaks of colonization and infection in critically ill, hospitalized patients.<sup>14</sup> One of the main problems facing by hospitals, clinicians and health care personnel in regards to *Acinetobacter baumannii* today is multidrug resistance. The objectives of the present study were to determine the incidence of multidrug resistant *Acinetobacter* infection and detection of the *adeB* gene in multidrug resistant and *bla<sub>NDM-1</sub>* gene in imipenem resistant *Acinetobacter baumannii*. We isolated 22 (6.37%) *Acinetobacter baumannii* strains from 345 wound swab samples. The findings are consistent with a study of Cevahir *et al* (2008) who studied wound swab samples and isolated 11.6% *Acinetobacter baumannii*.<sup>15</sup> The

**TABLE 1: Antimicrobial susceptibility pattern of isolated *Acinetobacter baumannii* (n = 22)**

Antimicrobial agent	Sensitive n (%)	Resistant n (%)
Amoxyclav (20/10 µg)	2 (9.09)	20 (90.91)
Ceftazidime (30 µg)	2 (9.09)	20 (90.91)
Cefotaxime (30 µg)	1 (4.55)	21 (95.45)
Ceftriaxone (30 µg)	4 (18.28)	18 (81.82)
Aztreonam (30 µg)	6 (27.27)	16 (72.73)
Imipenem (10 µg)	18 (81.82)	4 (18.18)
Ciprofloxacin (5 µg)	6 (27.27)	16 (72.73)
Gentamicin (10 µg)	6 (27.27)	16 (72.73)
Amikacin (30 µg)	8 (36.36)	14 (63.64)
Piperacillin (100 µg)	14 (63.64)	8 (36.36)
Colistin (10 µg)	22 (100.00)	0 (0.00)

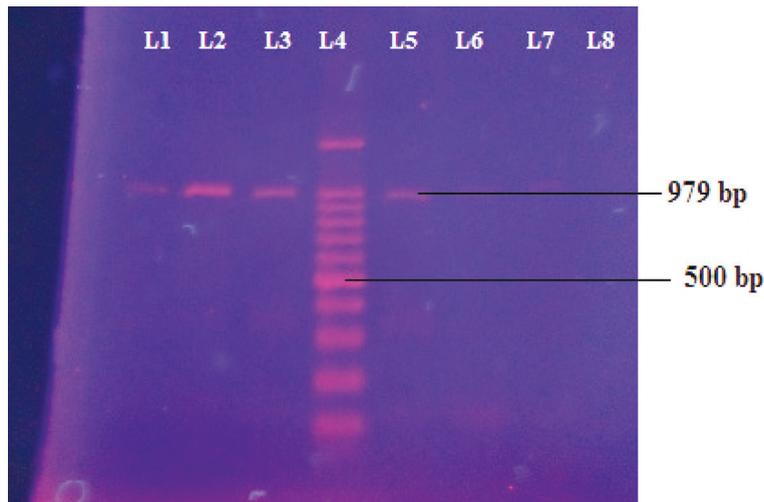


FIG. 1: Photograph of gel electrophoresis of amplified DNA of 979 bp for *adeB* gene (Lane 3 and 5). Hundred bp DNA ladder (Lane 4). Negative control *Escherichia coli* ATCC 25922 (Lane 6)

prevalence currently ranges from 2% to 10% of all gram-negative bacterial infections in Europe and about 2.5% in the United States.<sup>16</sup> There is a significant difference in the behaviour of this organism among isolates recovered from various geographic locations.<sup>17</sup>

Similar to other studies, most of the *Acinetobacter baumannii* were resistant to gentamicin (73%), ciprofloxacin (72.72%), ceftriaxone (81%).<sup>18,19</sup> In contrast, a previous study reported that 91% of the *Acinetobacter baumannii* was sensitive to ciprofloxacin. The antibiotic resistance in Bangladesh and developing countries commonly occurs due to

inappropriate antibiotic use, over-prescribing and inappropriate prescribing, unethical practices of health professionals, unqualified drug sellers offer alternative drugs when the prescribed drugs are not available.<sup>20</sup>

In the present study, no *Acinetobacter baumannii* was found resistant to colistin. This is probably due to less use of colistin. The absence of colistin resistance among *Acinetobacter baumannii* in this study permits the use of this antibiotic to treat the patients. Colistin related nephrotoxicity in 8-18% and neurotoxicity in 7-29% patients possess a significant challenge to the clinical utility of this drug.<sup>21</sup>

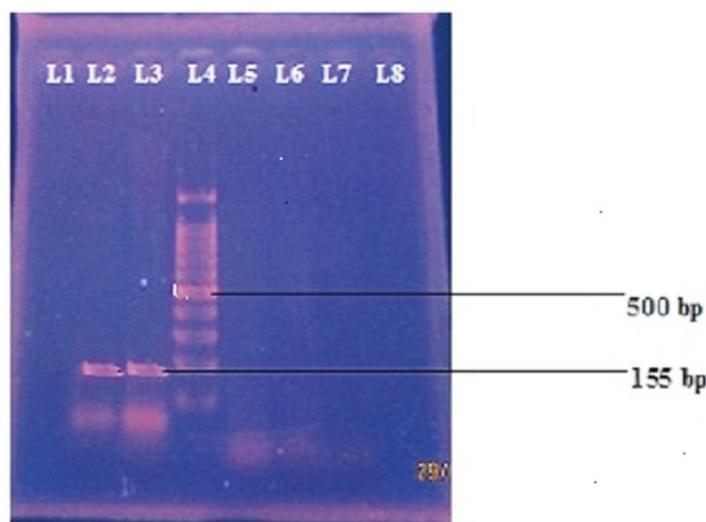


FIG 2: Photograph of gel electrophoresis for amplified DNA of 155 bp for *bla<sub>NDM-1</sub>* (Lane 2 and 3). Hundred bp DNA ladder (Lane 4). Negative control *Escherichia coli* ATCC 25922 (Lane 5)

In this study, the majority (81.81%) of the *Acinetobacter baumannii* strains were sensitive to imipenem. A study in India in 2009, showed 59% meropenem resistance among *Acinetobacter* spp. which reflect the evolving scenario in India.<sup>22</sup> High resistance percentages of *A. baumannii* strains to imipenem were reported by Joshi *et al* (2003) which was 71%.<sup>23</sup> Over the last 10 years, acquired resistance to imipenem has been increasingly reported worldwide in non-fermenting gram-negative bacilli (NFGNB) including *Acinetobacter* spp.<sup>24</sup>

In the present study, out of 22 *Acinetobacter baumannii* isolates, 2 (9.09%) were ESBL producers. In a previous study, 28% ESBL producers were detected among the studied *Acinetobacter* spp.<sup>25</sup> The higher prevalence of ESBL producers in Asia than in Europe and America was observed in a previous study.<sup>26</sup> Acquired extended-spectrum  $\beta$ -lactamase carriage occurs in *Acinetobacter* but is not as widespread as in *Klebsiella pneumoniae* or *Escherichia coli*.<sup>27</sup> If an isolate is confirmed as an ESBL producer by the CLSI recommended phenotypic confirmatory procedure, the isolate should be reported resistant to all penicillins, cephalosporins and aztreonam.<sup>9</sup> All the ESBL producing *Acinetobacter baumannii*, were sensitive to imipenem and colistin. Similarly in a study by Metan *et al* (2006), most (73%) of the ESBL producing *Acinetobacter baumannii* strains were found to be susceptible to imipenem.<sup>28</sup> Infection caused by ESBL producing organisms have currently been treated with carbapenems such as imipenem.<sup>29</sup>

In this study, 20 (90%) of the 22 isolated *Acinetobacter baumannii* were multidrug resistant. Dent *et al* (2010) reported 72% of the isolated *Acinetobacter baumannii* were multidrug resistant.<sup>30</sup> Most alarming in *Acinetobacter baumannii* infection is the ability of the organism to accumulate diverse mechanism of resistance to all commercially available antibiotics.<sup>31</sup> In the present study, 16 (80%) out of 20 multi-drug resistant *Acinetobacter baumannii* were positive for *adeB* gene. Previous studies showed that almost 90% of the multidrug resistant *Acinetobacter baumannii* are positive for *adeB* gene.<sup>5,6</sup> The presence of AdeABC multidrug efflux pump plays a major role in the development of antimicrobial resistance in *Acinetobacter* spp. The presence of either one or an interplay between these genes may have an effect on antimicrobial resistance in *Acinetobacter* spp.<sup>5</sup>

In this study, out of 4 imipenem resistant *Acinetobacter baumannii*, 2 (50%) were found positive for *bla*<sub>NDM-1</sub> gene. In a previous study, Farzana *et al* (2013) reported 22.8% *bla*<sub>NDM-1</sub> positive strain in imipenem resistant gram-negative bacteria in Bangladesh.<sup>32</sup> In the same study, 20% imipenem resistant *Acinetobacter baumannii* was *bla*<sub>NDM-1</sub> positive which is similar with the present study. MBL encoding genes have been detected from several gram-negative bacilli belonging to the family *Enterobacteriaceae* and also in *Acinetobacter* species.<sup>33</sup> Acquired MBLs in gram-negative bacteria are becoming an emerging resistant determinant worldwide.<sup>34</sup>

### Conclusion

The high incidence (91%) of multidrug resistance among the isolated *Acinetobacter baumannii* in our study highlights the emerging therapeutic challenge in Bangladesh. In this study 80% multidrug resistant *Acinetobacter baumannii* was positive for *adeB* gene. Among the isolates of *Acinetobacter baumannii* which were nonsusceptible to imipenem *bla*<sub>NDM-1</sub> gene was positive in 50% isolates. Early detection of this resistance mechanism, implementation of strict microbial policies and infection control programs may prevent the rapid dissemination of this organism.

### COMPETING INTERESTS

There is no conflict of interest.

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