Abstract

Introduction: Multidrug-resistant *Acinetobacter baumannii* is galloping, posing threat to tackle, and leaving us with limited options of treatment. **Methods:** The purpose of this study is to find the genotypic association in drug-resistant *A. baumannii* isolated from different sterile body fluids. Matrix-assisted laser desorption/ionization–time of flight confirmed *A. baumannii* isolates were taken and minimum inhibitory concentration (MIC) was determined by VITEK-2 AST system. The presence of resistance nodulation–division (RND)-efflux pump genes AdeABC-RS was detected by multiplex polymerase chain reaction. **Results:** Of the total 40 *A. baumannii*, 32 (80%) were multidrug resistant though all isolates were susceptible to Tigecycline. Similarly, 26 (81.25%) isolates were positive for RND-efflux pump genes AdeABC-RS. **Discussion:** RND efflux pump AdeABC-RS system plays a significant role in emerging multi drug resistant *A. baumannii*. Mutation in AdeS gene deciphers the role of regulatory gene. Hence, antimicrobial stewardship should be strictly followed and efflux pump inhibiting substances should be vigorously searched to bring back the era of existing antibiotics.

**Keywords:** *Acinetobacter baumannii*, regulatory system, resistance nodulation–cell division-efflux pump, sterile body fluids
range 0.5–4 µg/ml for colistin), were used as per CLSI guideline 2019. To observe the effect of efflux pump inhibitor (EPI) Carbonyl cyanide-m chlorophenylhydrazone (CCCP), it was added to Muller Hinton agar (MHA) Plate and final concentration of CCCP (Sigma Aldrich, USA) in MHA was adjusted 15 µg/ml.\(^\text{[4]}\) Bacteria were grown on MHA with CCCP and MIC was determined by VITEK-2 AST system. DNA was extracted from all the isolates by QIAamp DNA mini extraction Kit (Qiagen, USA) and multiplex polymerase chain reaction (PCR) was performed for detection of RND efflux pump gene AdeABC as well as Regulatory components AdeRS. The standard strains of \textit{A. baumannii} ATCC 19606 was used as positive control. Cycling condition used for multiplex PCR was, initial denaturation 94°C for 10 min, denaturation at 94°C for 1 min, annealing at 57°C for 30 s, extension at 72°C for 1 min and final extension was at 72°C for 7 min. Total numbers of cycle were 35. Agarose gel images of multiplex PCR product of RND efflux pump genes AdeBC and Regulatory genes AdeRS are shown in Figures 1 and 2.

Sequencing of 14 PCR products of four isolates were done, analyzed by Chromas version 2.6.4., and was deposited in NCBI for accession number. Accession numbers given were from MT912014-MT912027 for different genes of RND efflux pump.

**RESULTS**

The mean age of the patients was 40.27 ± 6.27 years (Min 3–Max 93 years). Total 40 isolates collected from different sterile body fluid and were confirmed by MALDI-TOF. Of the total, 14 (35%) from CSF, 14 (35%) from pleural fluid, 8 (20%) from Ascitic fluid and 4 (10%) from Peritoneal fluid were taken respectively. Of the total, 32 (80%) isolates were resistant to three or more drugs of different antimicrobial groups. Similarly, out of 32 isolates, 25 (78.13%) were extensively drug resistant. Among the MDR, 23 (71.87%) isolates were resistant to amikacin and gentamicin. In addition, the most effective antibiotic was tigecycline (No resistance, Food and Drug Administration interpretive criteria of tigecycline for \textit{Enterobacteriaceae} were used as EUCAST version 11.0, 2021 mentions insufficient evidence) followed by trimethoprim 14 (35%). For colistin, BMD was performed, out of 40 isolates, 3 isolates showed resistance to colistin in which MIC value was observed >4 µg/ml.

Similarly, due to effect of EPI, carbonyl cyanide 3-chlorophenylhydrazone (CCCP), 10 (25%) isolates showed 2–64 fold reductions in MIC values for different antibiotics. Change in MIC value after addition of EPI is shown in Table 1.

In case of colistin, out of 3 resistance strains, 8-fold reductions in MIC value were observed in 2 strains. Likewise, 26 (65%) isolates were positive for either of the AdeABC or AdeRS gene. AdeB was detected in most of the isolates, 15 (57.69%) followed by AdeA in 13 (50%). Sequencing analysis result showed mutation (Accession No. MT912022) at (L172P) position in RND–efflux pump regulatory gene AdeS.

**DISCUSSION**

Globally, it is confirmed from various reports that \textit{A. baumannii} is difficult to treat as it has various virulence factors and genes that is responsible for antimicrobial resistance. Our study showed 80% resistance to carbapenem (imipenem). Y Chen \textit{et al.}\(^\text{[4]}\) from china had showed 78.2% resistance to imipenem which is concurrent to our finding. Similarly, tigecycline was found most susceptible antibiotic, result is concordance with the finding of Y Chen \textit{et al.}\(^\text{[4]}\) and Lin L \textit{et al.}\(^\text{[5]}\) who found antibiotic susceptibility profile that advocates the limited drug choices in \textit{A. baumannii} therapy.

We have found decrease in MIC value of various antibiotics after addition of proton ionosphere compound CCCP. Similar reduction in MIC for various antibiotics has been reported in other studies.\(^\text{[6]}\) This infers the involvement of efflux system in these isolates showing high resistance to various antibiotics. RND efflux pump in bacteria is driven primarily by the physiological functions and not by the selective pressure imposed due to the use of antibiotics and are assumed to be highly conserved in bacterial genome. Few studies have considered sequence conservation of gene encoding efflux pump at the strain level.\(^\text{[7,8]}\)

Till date, over expression of RND systems i.e. AdeABC, AdeFGH and AdeUK has been associated with multidrug resistance in \textit{A. baumannii}. However, AdeABC strongly confers resistance to different group of drugs and reduced susceptibility to tigecycline.\(^\text{[9,11]}\) In our study, resistance up to 80% for most of the antibiotics was observed. Out of 32 MDR isolates, 26 (81.25%) were positive for AdeABC-RS system. Finding of this study is concurrent to the finding of Lin L \textit{et al.} that had shown AdeABC-RS in 70% of the

![Figure 1: Showing gel image of polymerase chain reaction product of AdeB and C gene. Well 1 showing 100 bp ladder, 2 Negative control, 3 A. baumannii ATCC 19606 positive control, 5 only AdeC gene, 6,7,10,12,14 B gene and well 13 showing AdeBC gene](image1)

![Figure 2: Showing gel image of polymerase chain reaction product of AdeRS gene. Well 1 100bp ladder, 2 A. baumannii ATCC 19606 positive control, 6 Negative control, 3, 4, 5, 7, 8, 9 and 10 AdeRS gene and 11, 12 showing only AdeS gene](image2)
Table 1: Multidrug resistant isolates of *Acinetobacter baumannii* showing 2-64 folds reduction in minimum inhibitory concentration value in the presence of an efflux pump inhibitor

| Antibiotics | RND-efflux pump and regulatory genes | Number of isolates with presence of efflux/regulatory genes and also change in MIC with EPI | Alteration in MIC value µg/ml in resistance strains after EPI (CCCP) | Total number of isolates with presence of efflux/regulatory genes and also change in MIC with EPI |
|-------------|-------------------------------------|-------------------------------------------------|--------------------|------------------------------------------|
|             |                                     |                                                 | MIC value before EPI-after EPI (S/I/R) | Total isolates with changed MIC |
| Tigecycline | AdeA, AdeB                           | 2                                               | I-S (4-2)                       | 2/4                                   | 4                           |
| Trimethoprim| AdeA, AdeB, AdeR and AdeS            | 2                                               | S-S (2-0.5)                      | 2/4                                   | 4                           |
|             | AdeB, AdeC AdeR and AdeS             | 5                                               | R-S (160-80)                     | 2/8                                   | 8                           |
|             | AdeA                                 | 1                                               | R-S (160-20)                     | 5/8                                   |                             |
| Imipenem    | AdeA, AdeB AdeR, AdeS                | 2                                               | R-S (8-4)                        | 2/6                                   | 6                           |
|             | AdeB AdeC                             | 1                                               | R-S (8-0.25)                     | 1/6                                   |                             |
|             | AdeA, AdeB, AdeR and AdeS            | 2                                               | R-S (8-0.5)                      | 2/6                                   |                             |
|             | AdeA                                 | 1                                               | R-S (16-0.25)                    | 1/6                                   |                             |
| Meropenem   | AdeA, AdeB, AdeR and AdeS            | 3                                               | R-S (16-0.25)                    | 3/3                                   |                             |
| Gentamicin  | AdeR, AdeS                           | 1                                               | R-S (16-4)                       | 1/4                                   |                             |
|             | AdeA                                 | 1                                               | R-S (16-2)                       | 1/4                                   |                             |
|             | AdeA, AdeR AdeS                      | 1                                               | R-S (8-4)                        | 1/4                                   |                             |
|             | AdeR, AdeS                           | 1                                               | S-S (4-1)                        | 1/4                                   |                             |
| Ciprofloxacin| AdeA, AdeB, AdeR and AdeS            | 2                                               | R-S (4-0.5)                      | 2/2                                   | 2                           |

S: Sensitive, I: Intermediate, R: Resistant, MIC: Minimum inhibitory concentration, EPI: Efflux pump inhibitor, CCCP: Carbonyl cyanide-m chlorophenylhydrazone

MDR isolates. It suggests a potential linkage between these genes and multidrug resistance. The strain, in which mutation was found, was MDR though susceptible to tigecycline. Many authors have depicted the mutation in AdeS gene in tigecycline-susceptible strains.\[12\]

**CONCLUSION**

*A. baumannii* have developed resistance to most of the commonly used antibiotics. RND-efflux pump has emerged as one of the newest bacterial resistance mechanism. Hence, new arsenal in the form of EPI need to be developed that will be useful to restore the fundamental action of antibiotics as well as to counteract the Global challenge of controlling the spreading of multi drug resistant *A. baumannii* strain.

**Research quality and ethics statement**

This study was approved by the Institutional Review Board / Ethics Committee (IEC 773). The authors followed applicable EQUATOR Network (“http:// www.equator-network.org/”) guidelines during the conduct of this research project.

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Nil.

**Conflicts of interest**

There are no conflicts of interest.

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