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Emergence of Plasmid Bearing *Acinetobacter baumannii* and *Pseudomonas aeruginosa* Isolates with High Multiple Antibiotic Resistance Index, India

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ABSTRACT

The emergence and spread of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* displaying multiple antibiotic resistance is of global concern. The current study aims to explore the MAR (multiple antibiotic resistance) indices of *A. baumannii* and *P. aeruginosa* clinical isolates and to determine the plasmidic resistance to antibiotics. Disc diffusion susceptibility test demonstrated resistance to 6 – 10 antibiotics among the gram-negative lactose non-fermenters: *A. baumannii* and *P. aeruginosa*, for which MAR indices ranged 0.6 – 1, using a panel of 10 test antibiotics. The bacterial isolates contained single plasmids of 'unstable nature' encoding resistance to 'ampicillin-chloramphenicol-tetracycline'. Monitoring plasmid mediated antibiotic resistance of *A. baumannii* and *P. aeruginosa*, in all defined local niches, ought to be an exigent mission in evading the global burden of antimicrobial resistance of such microorganisms.

Key words: *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, MAR index, Plasmidic antibiotic resistance.

INTRODUCTION

Two glucose-non-fermentative gram-negative bacteria, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, which belong to the ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* spp.) group (Ragupathi et al. 2019), are well recognized opportunistic pathogens displaying the capacity to cause nosocomial as well as community acquired infections (Navon-Venezia et al. 2005; Pang et al. 2019). Reports are available on the emergence of both *P. aeruginosa* and *A. baumannii*, having resistance to a number of antibiotics, such as aminoglycosides, fluoroquinolones and β-lactams (Garnacho-Montero and Jean-Francois 2019; Tacconelli et al. 2018), which constituted empirical therapies against such microorganisms. The World Health Organization (Leungtongkam et al. 2018) identified both of the microorganisms (*A. baumannii* and *P.*

aeruginosa) as the top priority pathogens, because of their aptitude in holding resistance to multiple antibiotics, requiring development of novel antimicrobial agents for an effective treatment. Plasmids play an important role in horizontal gene transfer among such bacterial populations in a defined niche, and thus help in the acquisition of multiple antibiotic resistance (MAR) by recipient strains from the donors. The plasmid mediated antibiotic resistances have been reported among different gram-negative pathogenic bacteria, including *A. baumannii* and *P. aeruginosa*, worldwide (Millan et al. 2018). The current study explores the MAR phenotypes of clinical *A. baumannii* and *P. aeruginosa* isolates in our part of the globe (Malda, West Bengal, India), and investigates R-plasmid among them (*A. baumannii* and *P. aeruginosa*).

MATERIALS AND METHODS

A total of five randomly selected clinical bacterial isolates: *A. baumannii* (n=2) and *P. aeruginosa* (n=3), were utilized in the current investigation. The antibiotic susceptibility testing was done by disc diffusion, following the interpretive criteria of the Clinical and Laboratory Standards Institute (CLSI, 2018), on Mueller–Hinton agar (Hi-Media, India) with 10 antibiotics (Hi-Media, India): ampicillin (Am: 10 µg/disc), chloramphenicol (Cm: 30 µg/disc), cefotaxime (Cf: 30 µg/disc), cefoxitin (Cx: 30 µg/disc), ciprofloxacin (Cp: 5 µg/disc), gentamicin (Gm: 10 µg/disc), imipenem (Im: 10 µg/disc), kanamycin (Km: 10 µg/disc), piperacillin (Pc: 10 µg/disc) and tetracycline (Tc: 30 µg/disc). The bacterial MAR phenotype was expressed in showing resistance to three or more antibiotics of different classes, and the MAR indices were determined and interpreted according to earlier publications (Krumperman 1983).

The plasmid instability among the bacterial isolates was performed through curing experiments, using SDS (sodium dodecyl sulfate), as explained earlier (Mandal et al. 2005). The plasmid DNAs from the test bacteria, *A. baumannii* (n=2) and *P. aeruginosa* (n=3), and the cured derivative strains were isolated following the protocol of Kado and Liu (1981). The isolated plasmids were subjected to agarose gel electrophoresis, in tris-borate buffer system (Maniatis 1982). The *Escherichia coli* V517 (≈54 kb) plasmid was used for the molecular size approximation of the isolated plasmids (in the gel), wherein the DNA bands were visualized and photographed using Gel Documentation system with ethidium bromide staining.

RESULTS AND DISCUSSION

The MAR phenotypes of the test microorganisms (*P. aeruginosa* and *A. baumannii*) as well as their MAR indices are represented in Table 1. The bacterial resistances to antibiotics of different classes (aminoglycosides, β-lactams. carbapenems and fluoroquinolones) have been found among bacteria in clinical settings (Tacconelli et al. 2018). As has been demonstrated by Sandhu et al. (2016), the *Acinetobacter* spp. isolates had MAR indices >0.2 – 1, for most of the pathogens tested, while as per the report of Davis and Brown (2016), most of the test *P. aeruginosa* isolates displayed MAR index of >0.2, with the mean value of 0.34 (range: 0.17–0.5). Herein, three isolates (*P. aeruginosa* PA1, *P. aeruginosa* PA3 and *A. baumannii* AB2) showed resistance to a panel of 10 antibiotics tested, thus having MAR index value of 1.0, while *A. baumannii* AB1 and *P. aeruginosa* PA2 had MAR indices 0.6 and 0.8, respectively (Table 1). The criteria as had been explained by the earlier author (Krumperman 1983) are interpretative to the origin of the *P. aeruginosa* and *A. baumannii* clinical isolates pertinently from the places with high antibiotic load.

The plasmid profile of *P. aeruginosa* and *A. baumannii* are depicted in Fig. 1. Along with the bacterial intrinsic resistance to antibiotics, resistance acquisition due to HGT (via plasmids) have been reported among pathogenic bacteria including *P. aeruginosa* and *A. baumannii* (Ragupathi et al. 2019; Pang et al. 2019; Leungtongkam et al. 2018; Millan et al. 2018). In the instant investigation, the bacterial isolates displayed single plasmid that co-migrated with ≈54 kb plasmid molecular marker from *E. coli* V517. Loss of a common resistance to 'Am-Cm-Tc', among all the *P. aeruginosa* and *A. baumannii* AB2 isolates, along with the loss of the plasmid, following curing with SDS treatment occurred (Fig. 1), which suggest the plasmidic 'Am-Cm-Tc'-resistance of the test bacteria; the *A. baumannii* AB1 cured derivative became sensitive Am and Tc.

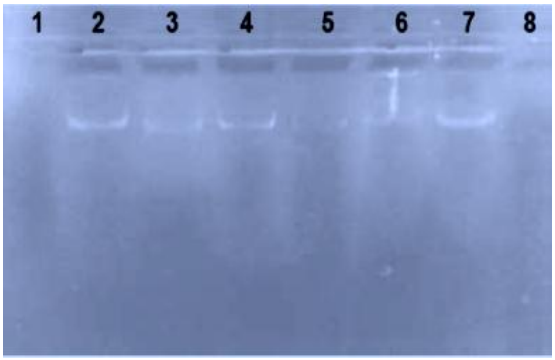

Fig. 1. Plasmid profile of clinical bacterial isolates; lane 1: *A. baumannii* AB2 (cured), lane 2: *E. coli* V517 (≈54 kb plasmid), Lane 3: *A. baumannii* AB2; lane 4: *A. baumannii* AB1, lane 5: *P. aeruginosa* PA1, lane 6: *P. aeruginosa* PA2, lane 7: *P. aeruginosa* PA3, lane 8: *P. aeruginosa* PA3 (cured); the cured strains did not display any plasmid (with concomitant loss of antibiotic resistance to Am, Cm and Tc).

Earlier, the plasmid mediated multiple antibiotic resistance has been reported in another gram-negative opportunistic pathogen, *K. pneumoniae*,

which has also been recognized as a member of ESKAPE group of bacteria, causing serious nosocomial and community acquired infections (Ramirez et al. 2014). Thus, plasmid mediated multiple antibiotic resistance, among the *P. aeruginosa* and *A. baumannii* clinical isolates, is of global occurrence.

Table 1. The MAR phenotypes and MAR indices of clinical bacteria.

Bacteria	MAR phenotypes	MAR index	Resistance
P. aeruginosa PA1	Am-Cm-Tc-Im-Cf-Cx-Cp-Pc-Ak-Gm	1	10-drug
P. aeruginosa PA2	Am-Cm-Tc-Im-Cf-Cx-Pc-Ak	0.8	8-drug
P. aeruginosa PA3	Am-Cm-Tc-Im-Cf-Cx-Cp-Pc-Ak-Gm	1	10-drug
A. baumannii AB1	Am-Tc-Im-Cf-Cx-Ak	0.6	6-drug
A. baumannii AB2	Am-Cm-Tc-Im-Cf-Cx-Cp-Pc-Ak-Gm	1	10-drug

MAR; multiple antibiotic resistance, Am; ampicillin, Cm; chloramphenicol, Cf; cefotaxime, Cx; cefoxitin, Cp; ciprofloxacin, Gm; gentamicin, Im; imipenem, Km; kanamycin, Pc; piperacillin, Tc; tetracycline.

CONCLUSION

The current findings suggest that *P. aeruginosa* and *A. baumannii* harboring R-plasmid have been established in the local niches of this part of the globe (West Bengal, India), because of the selective pressure of antibiotics of varied classes, readily showing resistance to multiple antibiotics.

Conflict of interest: There was no conflict of interest.

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