

Molecular characterization of carbapenem-resistant *Acinetobacter baumannii* strains from a tertiary care center in South India

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Abstract

Objectives: Carbapenem resistant *Acinetobacter baumannii* is an important therapeutic and infection control challenge worldwide. In this study, we investigated the prevalence and distribution of molecular mechanisms of resistance among carbapenem resistant *A. baumannii* species at a tertiary care setting in South India.

Materials and Methods: A total of 89 non-duplicate clinical isolates of carbapenem-resistant *A. baumannii* were collected from critical care units of St. John's Medical College Hospital, Bengaluru, India. Polymerase chain reaction (PCR) was performed to detect *bla*_{OXA} type carbapenemase *bla*_{OXA-51}-like, *bla*_{OXA-23}-like, *bla*_{OXA-24}-like and *bla*_{OXA-58}-like, MBL genes *bla*_{NDM}, *bla*_{IMP} and *bla*_{VIM} genes. Molecular typing of carbapenem-resistant *A. baumannii* strains was performed by using Rep-PCR.

Results: Eighty-seven of the isolates were found to carry the *bla*_{OXA-51} gene and 81 (91%) isolates were found to have *bla*_{OXA-51}-like gene and *bla*_{OXA-23} gene. The *bla*_{OXA-24}-like gene was detected in two isolates of which one also carried *bla*_{OXA-51}-like and one isolate carried *bla*_{VIM} coding gene. The prevalence of *bla*_{NDM}, *bla*_{IMP} and *bla*_{VIM} genes was 12(13%), 14 (16%) and 57(64%) respectively. Cluster analyses revealed a 90% similarity and were divided into 5 clusters. Most of the isolates containing carbapenemases coding genes grouped under cluster A, C and UC. Considerable heterogeneity was observed within UC cluster indicating circulation of multiple strains of *A. baumannii* within our institution.

Conclusions: Carbapenemase coding *bla*_{OXA-23}, *bla*_{OXA-24} and *bla*_{OXA-51}-like were more common than *bla*_{VIM} and *bla*_{NDM}. The presence of *bla*_{NDM} with other genes coding for carbapenemases indicate the ability of the strains to acquire novel genes despite having its share of the *bla*_{OXA} like carbapenemase.

Keywords: *Acinetobacter baumannii*; carbapenemases; Genomic diversity; Rep-PCR; carbapenem resistance

Caracterización molecular de cepas de *Acinetobacter baumannii* carbapenem-resistente en un centro de atención terciaria en el sur de la India

Resumen

Objetivos: El *Acinetobacter baumannii* resistente a Carbapenem es un reto importante en todo el mundo para su tratamiento y para el control de infecciones hospitalarias. Nosotros estudiamos la prevalencia y los mecanismos de resistencia en aislados de un centro de atención terciario, en el sur de la India

Materiales y Métodos: Se estudiaron 89 aislados clínicos de *A. baumannii* recolectados en unidades de cuidado crítico del Hospital St. John's Medical College en Bengaluru, India. Se realizó amplificación por PCR (Reacción en Cadena de Polimerasa) y luego tipificación molecular con la técnica Rep-PCR (PCR de elementos repetitivos palíndricos) para detectar los genes de carbapenemasa *bla*_{OXA}, *bla*_{OXA-51}, *bla*_{OXA-23}, *bla*_{OXA-24}, *bla*_{OXA-58}, MBL, *bla*_{NDM}, *bla*_{IMP} y *bla*_{VIM}.

Resultados: Se encontraron 87 aislados que llevaban el gen *bla*_{OXA-51} y de ellos en 81 (91%) se encontró *bla*_{OXA-51} y *bla*_{OXA-23}. El *bla*_{OXA-24} se detectó en dos aislados de los cuales uno de ellos llevaba *bla*_{OXA-51} y otro *bla*_{VIM}. Los genes *bla*_{NDM}, *bla*_{IMP} y *bla*_{VIM} se encontraron en 12 (13%), 14 (16%) y 57(64%) de los aislados, respectivamente. El análisis de agrupamiento reveló un 90% de similitud entre los aislados y que podían asignarse a 5 agrupamientos. La mayoría de aislados llevaban genes de carbapenemasas de los grupos A, C y UC. Se observó mucha heterogeneidad dentro del agrupamiento UC indicando que existe circulación de múltiples cepas de *A. baumannii* dentro de nuestra institución.

Conclusiones: Las carbapenemasas que codifican para *bla*_{OXA-23}, *bla*_{OXA-24} y *bla*_{OXA-51} son más comunes que *bla*_{VIM} y *bla*_{NDM} en nuestra institución. La presencia de NDM con otros genes codificando para carbapenemasas indica la capacidad que tienen este tipo de aislados para adquirir nuevos genes a pesar de contar con *bla*_{OXA}.

Palabras clave: *Acinetobacter baumannii*; carbapenemasas; diversidad genómica; Rep-PCR; resistencia a carbapenem

Introduction

Acinetobacter baumannii (*A. baumannii*) a non-glucose fermenting Gram-negative bacillus, is an important cause of nosocomial infections and widely isolated from healthcare facilities, especially in the intensive care units (ICUs). They are increasingly being associated with increased morbidity and

mortality among hospitalized patients. The increased use of invasive procedures and exposure to antibiotics increases the risk of infections among immunocompromised patients admitted in critical care units. These organisms are known to colonize various surfaces of the environment and patients admitted to healthcare institutions. As they are known to survive in unfavorable environments and can contaminate

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Recibido: 11/05/2018; Aceptado: 24/02/2019

Cómo citar este artículo: B.S. Kalal, et al. Molecular characterization of carbapenem-resistant *Acinetobacter baumannii* strains from a tertiary care center in South India. Infectio 2020; 24(1):27-34

various surfaces present in the health care units^{1,2}. The ability to adapt and survive in an unfavorable environment, potential to acquire multiple genes for antibiotic resistance and to colonize the patient has favored the organism to be transmitted easily and cause prolonged and repeated outbreaks^{3,4}.

Carbapenems were considered the most powerful antibiotics because of their extremely effective antibacterial activity and low toxicity; however, with the emergence of carbapenem resistance, *A. baumannii* has become a growing therapeutic concern worldwide^{5,6}. Over the last 15 years, the resistance of *A. baumannii* to expanded-spectrum cephalosporins and carbapenems has grown rapidly⁷. Carbapenem resistance, among these pathogens, is attributed to reduced expression of outer membrane proteins (29 kDa, 33-36 kDa), change of the penicillin-binding proteins, the specific drug efflux and carbapenemase production. Carbapenem-hydrolyzing Class D oxacillinases (*bla*_{OXA-23} to 27, 40, 51, and 64 to 71) are the predominant carbapenemase has been reported globally (8, 9). The *bla*_{OXA-51} is thought to be probably intrinsic to the genetic makeup of the organism. However, reports on the *bla*_{IMP} or *bla*_{VIM} class Metallo-β-lactamase (MBL such as *bla*_{IMP-1}, 2, 4 and 5, and *bla*_{VIM-1} and *bla*_{VIM-2}) producing *A. baumannii* are also on the rise (10). Although most *bla*_{NDM} genes are frequently reported in Enterobacteriaceae, they have also been detected in *Acinetobacter* species, first of such case of *bla*_{NDM} in *Acinetobacter* was reported in 2010 from India(11). Expression of more than one type of carbapenemases has been documented in India^{12,13}. Due to the ability to survive in a harsh environment (presence of disinfectants, exposure to antibiotics), these organisms are known to persist and are able to be transferred to different healthcare facilities by colonizing or infecting patients. Prevention of infections by these organisms is a tough challenge. Outbreaks and persistent presence of these organisms have been documented in intensive care units for prolonged periods¹³. The antibiogram patterns may not be able to suggest similarity among the different strains in circulation during a particular period.

A. baumannii strains have been typed using molecular methods such as ribotyping, amplified fragment length polymorphism, restriction fragment length polymorphism, and repetitive extragenic palindromic sequence-based polymerase chain reaction (Rep-PCR)¹⁴. Due to limited gene set or technical limitations, most new tests are not suitable for clinical routine monitoring in low prevalence settings. Rep-PCR is simple, rapid test that can be highly discriminatory for the identification and differentiation of *A. baumannii*¹⁵.

The study was aimed to describe the presence of carbapenemases coding genes among Meropenem/Imipenem resistant *A. baumannii* obtained from clinical samples by phenotypic methods and to understand their clonal relationship using Rep-PCR.

Materials and Methods

A total of 89 non-duplicate isolates of meropenem/ Imipenem resistant *A. baumannii* were collected. These organisms were isolated from the samples received from patients in the critical care units (intensive care, burns, post-operative areas) of St. John's Medical College Hospital, Bengaluru, India between May 2011 and January 2012. The isolates were collected from blood, respiratory secretions sputum, urine, pleural fluid, ascitic fluid, and wounds, and were considered nosocomial if the specimens had been obtained more than 48 hours after the time of patient's admission. More than one isolate from the same patient was not included. *A. baumannii* was identified in the clinical microbiology laboratory by standard biochemical tests as well as the Vitek 2 system (bioMérieux, Durham, NC), when necessary. Those identified *A. baumannii* complex were further confirmed *A. baumannii* using a multiplex PCR method by using the specific *Acinetobacter baumannii* primers (P-Ab-ITSF and P-Ab-ITSB) and the internal control primers (P-rA1 and P-rA2) specific for the *recA* gene of all *Acinetobacter* spp (16). The study was approved by the Institutional Ethical Board, St. John's Medical College, Bengaluru.

Susceptibility testing

Antibiotic susceptibility was determined by the standard agar disc diffusion method using Mueller–Hinton agar plates (Himedia, India) using antimicrobial agents and interpreted according to the guidelines of the Clinical and Laboratory Standards Institute 2012 (CLSI, 2012)¹⁷. The following antibiotics Gentamicin (Gen), tetracycline (Tetra), trimethoprim-sulphamethoxazole (Cotri), cephalixin (Ceph), ciprofloxacin (CFX), amikacin (Amik), Ceftriaxone and Tazobactam (Omna), cefotaxime, netilmicin (Net), ceftazidime (Fort), cefaperazone (CpZ), piperacillin (Pip); piperacillin/tazobactam (PT), Meropenem and imipenem (Imi/Mero) were tested using discs obtained from Himedia labs, Mumbai, India. Meropenem and imipenem were obtained from BD India Ltd. Resistance to Meropenem was confirmed using agar dilution method. A minimum inhibitory concentration (MIC) of ≥ 16 µg/mL for imipenem and meropenem was used to define resistance. *Pseudomonas aeruginosa* ATCC 27853 was used as reference strains for quality control of the antibiotic discs and agar dilution.

Isolation of bacterial DNA

The bacterial genomic DNA was prepared by using the lysis buffer (1% Triton X-100, 10 mM Tris pH 8.0, 1 mM EDTA) and boiling method. Briefly, colonies were picked from a MacConkey plate and inoculated into Luria-Bertani broth (Himedia, Mumbai, India) and incubated at 37 °C in a shaker incubator for 18 – 24 hrs. After the incubation, cell pellet was prepared from the broth. The cell pellet was washed in molecular grade water and lysis buffer was added. The bacterial suspension was heated at 95 °C for 15 min, and then pelleted by centrifugation at 12,000 ×g for 5 min. The supernatant was transferred to fresh sterile 1.5 ml tubes, for use as templates for PCR amplification¹⁸.

PCR analysis of carbapenemases-encoding genes

The genes detected included the integrase gene *intl*, OXA-type carbapenemase gene clusters *bla*_{OXA51-like}, *bla*_{OXA-23-like}, *bla*_{OXA-24-like} and *bla*_{OXA-58-like}, MBL genes *bla*_{NDM}, *bla*_{IMP} and *bla*_{VIM}¹⁹⁻²¹. The negative control was run with every PCR. The primers used for the PCR are listed in Table 1²² and PCR conditions are per the references quoted. The PCR products were separated on 2% agarose gels by electrophoresis, stained with ethidium bromide at a final concentration of 0.5 µg/ml, and then visualised under the gel documentation system (Biorad Life Science, CA, USA).

Rep-PCR fingerprinting

The repetitive extragenic palindromic polymerase chain reaction (Rep-PCR) fingerprinting was performed as previously described primer pair Rep-1 (5'-IIIGCGCCGICATCAGGC-3') and Rep-2 (5'-ACGTCTTATCAGGCCTAC-3')^{23,24} and are listed in Table-1. PCR products were analysed by electrophoresis on a 1,5% agarose gel and stained with ethidium bromide (5 mg/L). Strains were considered to be highly similar and belonging to the same DNA group when all visible bands of two isolates had similar migration distance. Within the same Rep-type, strains differing by no more than two bands were considered subtypes. Gel images were subsequently analysed by Bionumerics software (Applied Maths, Kortrijk, Belgium) A dendrogram was generated to analyse the similarity between the isolates, using the unweighted pair group with arithmetic averages (UPGMA) and the Dice similarity coefficient with 1% of tolerance and 0.5% of optimization²⁵.

Results

Bacterial isolates and antimicrobial susceptibility pattern

The isolates were predominantly from respiratory secretions (endotracheal aspirates or sputum) 60 (67.4%), followed by pus 25(28.1%), urine 10(11.2%) and three from other body fluids and two from blood samples. Forty-nine isolates (55%) were from male patients. As seen in Figure-1, most of the isolates were resistant to nearly all antibiotics tested except for netilmicin. Resistance to at least one of aminoglycoside or β-lactam antibiotics were observed in 80% and 97% of the strains. A total of 36 (40%) isolates out 89 are resistant to all the antibiotics tested and rest of the 53 isolates were multi-drug resistant. Minimum inhibitory concentrations (MIC) for meropenem and imipenem determined using the agar dilution method (CLSI 2010).

Detection and characterization of antibiotic resistance coding genes

As seen in Table – 2, the majority of carbapenem-resistant isolates carried *bla*_{OXA-51} (98%) and *bla*_{OXA-23} (96%) genes followed by *bla*_{VIM} gene (67%). Class-1 integrase genes were found in all of *A. baumannii* strains tested (n=89/89,100%) whereas class 2 integrase genes were found in 5 (n=5/89, 6%) isolates. A total of 14 isolates out of 89 carries *bla*_{NDM-1} gene. The association of ISAb_a-1 along with *bla*_{OXA-23-like} and *bla*_{OXA-51-like} was present in 92% and 94% respectively.

Rep-PCR

Rep-PCR showed considerable diversity of genotypes among the isolates examined. The isolates were grouped into five different clusters A to E. However, there are many isolates which has not shown any bands or showed one or two bands categorized as unclassified (UC). The clusters A to C was defined by using a similarity threshold of 90% were marked in the dendrogram (Figure 2). Majority of the isolates from all the clusters demonstrated the presence of *bla*_{OXA-23}, *bla*_{OXA-51} and class-1 integron coding genes. Similarly, isolates in Cluster B carried *bla*_{VIM} in few isolates, *bla*_{VIM} coding gene coexisted with *bla*_{IMP} and one isolate carried both *bla*_{NDM} and *bla*_{IMP} coding genes. The Clusters D to E was defined by using a similarity threshold of 90% as marked in the dendrogram. Isolates in cluster D in addition to *bla*_{OXA-23}, *bla*_{OXA-51} and class-1 integrons coding genes, demonstrated only the presence of *bla*_{VIM} gene. The isolates grouped under Cluster E carried genes code for *bla*_{VIM}, *bla*_{IMP} and *bla*_{NDM}.

Discussion

Reports on carbapenemase - producing *A. baumannii* isolates are rising globally due to increased carbapenem use thereby creating a selection pressure. In the current study, we found that most of the carbapenem-resistant isolates (67%) were from respiratory secretions. Outbreaks caused by *A. baumannii* strains have been reported in the ICU due to cross-contamination or spread among patients^{1,2,6,27}. It has been reported that carbapenem resistance rates in *A. baumannii* have mostly exceeded 40% throughout all of India. All of the isolates tested in the present study showed a high level of resistance to majority of the antibiotics except netilmicin. Prashanth et al., report the high effectiveness of netilmicin against MDR *A. baumannii* from India²⁸. However, Saranathan et al., also reported 48% of netilmicin resistance among *A. baumannii* clinical isolates from Southern India²⁹. Infections like bacteremia and ventilator-associated pneumonia caused by XDR *A. baumannii* result in >50% mortality rates³⁰. In the present study, 80% of *A. baumannii* isolates were detected as XDR. Rynga et al., report 78% XDR *A. baumannii* clinical isolates from two hospitals from New Delhi, India³¹. Similarly, Maspi et al., report 71% of XDR *A. baumannii* isolates from Iran³². Multi-drug resistant *A. baumannii* has caused many nosocomial outbreaks worldwide, and is considered as a difficult to treat nosocomial pathogen due to resistance to many antimicrobial agents and has been increasingly associated with mortality^{10,13}.

The higher percentage of oxacillinases, *bla*_{OXA-23} and *bla*_{OXA-51} (most common resistance genes), than metallo-β-lactamases (*bla*_{VIM} and *bla*_{NDM}) detected from the tested *A. baumannii* isolates was in accordance with earlier published reports from India^{12,33}. However, in the present study, *bla*_{OXA-58-like} carbapenem resistance coding genes was not detected from any of the tested isolates. The *bla*_{OXA-58} like genes have been more commonly reported from Europe, North and South America, and West Asia, however low prevalence (2%) has been re-

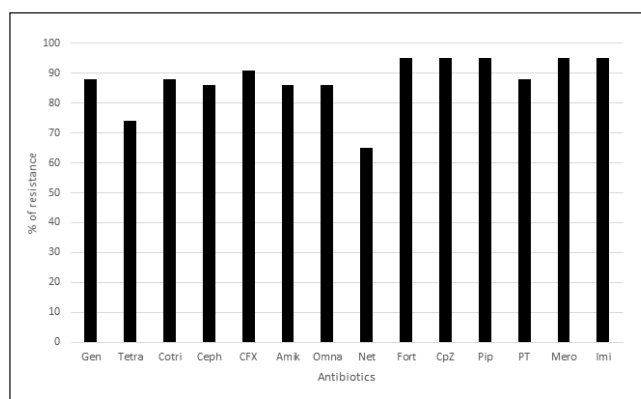


Figure 1. In- vitro antibiotic susceptibility profile of *Acinetobacter baumannii* species clinical isolates

Note: Antibiotics tested is Gentamicin (Gen), tetracycline (Tetra), trimethoprim-sulphamethoxazole (Cotri), cephalixin (Ceph), ciprofloxacin (CFX), amikacin (Amik), Ceftriaxone and Tazobactam (Omna), cefotaxime, netilmicin (Net), ceftazidime (Fort), cefaperazone (CpZ), piperacillin (Pip); piperacillin/tazobactam (PT), Meropenem and imipenem (Imi/Mero).

ported from South India³⁴. In this study we observed, both bla_{OXA-51} gene and bla_{OXA-23} were important in conferring resistance to carbapenems among 91% of the isolates which was comparatively higher to other Indian studies³⁴. In our study, approximately 93% of the imipenem/carbapenem-resistant isolates carried the combination of bla_{OXA-51} and bla_{OXA-23} along with insertion sequences ISAbA1. Vijayakumar et al., reported the association of ISAbA1 and bla_{OXA-23} gene among Carbapenem resistant *A. baumannii* from a Tertiary Care Hospital in South India³⁵. However, Ganjo et al., report the bla_{OXA-23} gene in combination with ISAbA1 insertion element was largely responsible for carbapenem resistance in clinical *A. baumannii* isolates from Kurdistan, Iraq³⁶. Present study report 11/12 bla_{NDM} and bla_{OXA-23} coding gene carried isolates associated with ISAbA1. Karthikeyan et al., reported the co-existence of bla_{NDM} gene and bla_{OXA-23} with ISAbA1 from India¹¹. A total of 14/89 isolates carried bla_{IMP} coding gene, out of these 14 isolates, four isolates carried bla_{NDM} and bla_{OXA-23} associated with ISAbA1. However, the coexistence of bla_{NDM-1} with the bla_{OXA-23} and bla_{IMP} among *A. baumannii* isolates was reported from China³⁷. Carbapenem resistance in Europe is mostly due to the occurrence of bla_{OXA} carbapenemases, however, the bla_{IMP} and bla_{VIM} MBLs are more prevalent in the Far East. Present study detects bla_{VIM} Metallo- β -lactamses coding genes from 64% of *A. baumannii* isolates, among these 7/89 (8%) co-existed with bla_{NDM} . Rhamanet al., reported bla_{VIM} was significantly associated with bla_{NDM} genes in *A. baumannii* isolates from India³⁸.

Incretion Sequence elements have significant role in *A. baumannii* antimicrobial resistance. ISAbA1, ISAbA2, ISAbA3, ISAbA4 and IS18 are frequently associated with the expression of carbapenemases genes in *A. baumannii*. The present study observed a prevalence of 94% and 92% of ISAbA1 elements associated with bla_{OXA-51} -like and bla_{OXA-23} -like genes respectively. Similarly, Villalón et al. (2013) investigated the presence of IS elements in 59 multidrug-resistant *A. baumannii* isolates and observed a prevalence of 93.2% of ISAbA1 from Spain.

It is important to note that IS elements such as ISAbA1 can contribute to the spread of carbapenemase genes among different *Acinetobacter* species³⁹. The high prevalence of class 1 integrons among multidrug-resistant *A. baumannii* clinical isolates has been confirmed worldwide. The epidemic potential of *A. baumannii* may be linked to the presence of class-1 integrons and ISAbA1. In our study, class-1 integrons was present in all of *A. baumannii* isolates. Whereas, class 2 integrons were present in four isolates and co-existed with class -1 integron. In the present study, 93% of *A. baumannii* isolates carried class-1 integrons coding genes and these isolates co-existed with ISAbA1. Sun et al report 64% of the class -1 integrons in *Acinetobacter* spp. isolates in northeastern China⁴⁰. Amin et al reported the class -1 integron is often responsible for the dissemination of the MBL genes among *A. baumannii* isolates from Iran⁴¹. In the present study, 83% (74/89) of *A. baumannii* isolates were positive for class-1 integrons coding genes.

This study was also aimed to determine the distribution of resistant genes in genetically similar and dissimilar carbapenem-resistant *A. baumannii* strains by using Rep-PCR. All the isolates were classified into different clonal clusters A to E according to genetic similarity based on banding pattern obtained by Rep-PCR. Resistance coding genes detected from these isolates were not associated with a particular group; rather each group confined more than one resistance gene type. Similar to other study, most of the strains showed to harbor multiple carbapenemases genes possibly indicate that there are multiple clones in circulation in our hospital. In this study, it has been shown that genetic determinants like bla_{OXA-23} , bla_{OXA-51} , $intl1$, ISAbA1, bla_{VIM} , bla_{NDM} , and bla_{IMP} were widely distributed among all the carbapenem resistant *A. baumannii* isolates grouped under different clonal clusters A to E. These genetic determinants are capable of possessing the potential horizontal gene transfer between susceptible *A. baumannii* or any other bacterial species. Rep-PCR uses amplification of intervening fragments between highly conserved sequences by means of consensus primers^{14,15}. It is a rapid, simple and low-cost method for molecular typing, which has been applied previously to identify genetic differences among the isolates of *A. baumannii*¹⁵. It is challenging to discriminate a clear pattern among some isolates because rep-PCR bands are faint, and some profiles have few bands. The rep-PCR is less discriminatory than PFGE in identifying unique strains. However, sometime it requires isolates sharing the same pattern to be tested with a more discriminatory method, like PFGE or multi-locus sequence typing whenever appropriate. Vijayakumar et al reported the carbapenem-resistant *A. baumannii* isolates were grouped under clonal complex 208 which belongs to the international clonal lineage 2 and high occurrence of ST-848 carrying bla_{OXA-23} -like gene from India⁴².

There has been increasing concern regarding the rise of *Acinetobacter* infections in critically ill patients. Our study also showed that *Acinetobacter* infections most frequently involved the respiratory tract of intubated patients as also reported

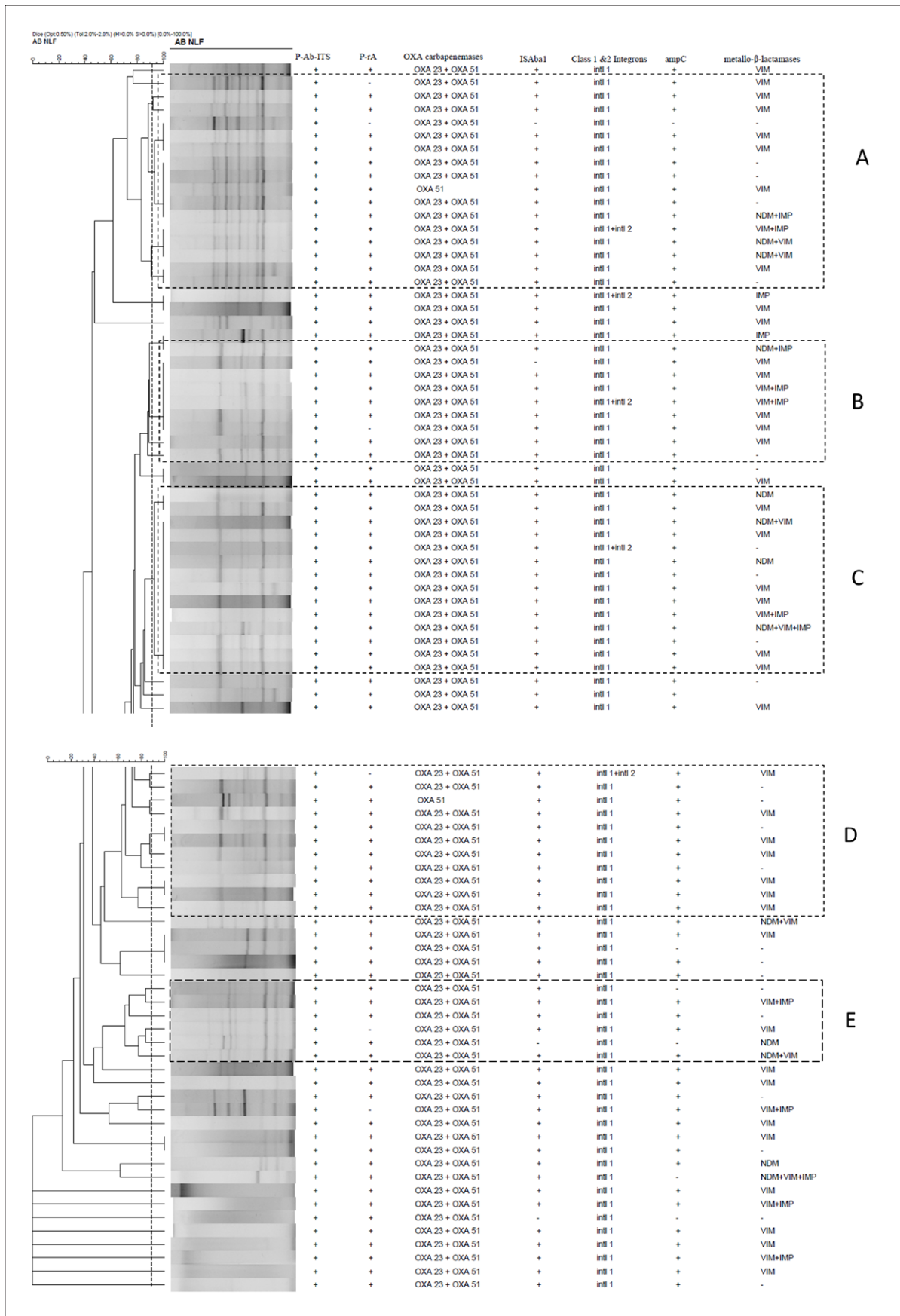


Figure 2. Repetitive sequence-based polymerase chain reaction (rep-PCR) similarity analysis and gel images for multidrug-resistant *Acinetobacter baumannii* isolates.

Table 1. Primers used for amplification of resistance genes by polymerase chain reaction

Genes	Primer sequence	Reference
OXA51-LIKE F	TAATGCTTTGATCGGCCTTG	21
OXA51-LIKE R	TGGATTGCACCTTCATCTTGG	
OXA 23-LIKE F	GATCGGATTGGAGAACCAGA	21
OXA 23-LIKE R	ATTCTGACCGCATTTCAT	
OXA 24-LIKE F	GGTTAGTTGGCCCCCTTAAA	21
OXA 24-LIKE R	AGTTGAGCGAAAAGGGGATT	
OXA 58- F	AAGTATTGGGGCTTGTGCTG	21
OXA 58- R	CCCCTCTGCGCTCATACATAC	
IMP F	CATACWTCGTTYGAAGAAGTTAACGG	21
IMP R	GAGAATTAAGCCACTCTATTGC	
VIM F	ACGCACCTTTCATGACGACCCGCG	21
VIM R	TTGTCGGTCGAATGCGCAGCACC	
NDM F	GGTTGGCGATCTGGTTTTTC	23
NDM R	CGGAATGGCTCATCACGATC	
ISAbA-F	CACGAATGCAGAAGTTG	22
ISAbA-R	CGACGAATACTATGACAC	
Int1-F	CAGTGGACATAAGCCTGTTC	22
Int1-R	CCCAGGCATAGACTGTA	
Int2-F	TTGCGAGTATCCATAACCTG	22
Int2-R	TTACCTGCACTGGATTAAGC	
Rep-1	IIIGCGCCGICATCAGGC	25
Rep-2	ACGCTTATCAGGCCTAC	

by others⁴³. The ICU and other critical care areas provide an ideal environment for the persistence of this organism in the presence of intense antibiotic exposure, enhancing mutation or acquisition of additional resistance mechanisms. The ability of this organism to acquire resistance from other gram-negative bacilli has been demonstrated by the presence of the bla_{NDM} gene, first reported from Enterobacteriaceae³. Multiple hospital admissions and visit to different institutions increases the risk of colonization with different strains of the pathogen³³. Despite appropriate infection control precautions these organisms have been reported to cause prolonged outbreaks among patients admitted in the ICUs²⁶.

Table 2. Distribution of resistance-associated genes among clinical isolates of multidrug-resistant *Acinetobacter baumannii*

Clonal groups	Inta I	Inta II	ISAbA1	blaOXA-51-like	blaOXA-23-like	NDM	VIM	IMP	ISAbA1 + blaOXA-51-like	ISAbA1 + blaOXA-23-like	ISAbA1 + NDM	ISAbA1 + VIM	ISAbA1 + IMP
A (n=16)	16(100)	1(6.2)	15(94)	15(94)	15(94)	3(20)	10(67)	2(13)	15(94)	15(94)	3(20)	10(67)	2(13)
B (n=9)	9(100)	1 (11)	9(13)	9(100)	8(13)	1(11)	7(64)	3(33)	9(100)	9(100)	1(11)	7(64)	3(33)
C (n=14)	14(100)	1 (7)	14(100)	14(100)	14(100)	4(28)	10(71)	2(14)	14(100)	14(100)	4(29)	10(71)	2(14)
D (n=11)	11(100)	1 (9)	11(100)	11(100)	9(82)	0	7(64)	0	11(100)	9(82)	0	7(64)	0
E (n=6)	6(100)	0 (0)	5(83)	6(100)	6(100)	2(33)	3(50)	1(17)	5(83)	6(100)	1(17)	3(50)	1(17)
UC (n = 33)	33(100)	1 (3)	30(91)	32(97)	30(91)	2(6)	20(61)	6(61)	30(91)	29(88)	2(6)	18(54)	6(18)
Total (n=89)	89(100)	5(6)	84 (93)	87 (98)	85 (96)	12(13)	57(64)	14 (16)	84 (94)	82 (92)	11 (12)	55 (62)	14 (16)

Conclusion

A. baumannii previously thought to be contaminants have now gained notoriety as multidrug resistant, hospital-acquired pathogens due to the acquisition of a number of resistance genes. Analysis of the genes that codes for carbapenem resistance among randomly selected isolates has confirmed the presence of multiple resistant genes such as, bla_{OXA-23}, bla_{OXA-51}, and bla_{VIM} and isolates that showed considerable genetic heterogeneity. Presence of bla_{NDM} was documented along with other Metallo-β-lactamases genes. In most of isolates the combination of genes demonstrates the ability of the organism to acquire novel genes from other bacteria (such as bla_{NDM}) and increase its own repertoire of resistant genes, thereby markedly reducing treatment options with β-lactam antibiotics.

Acknowledgement

We gratefully acknowledge the grant support of the Research Society of St. John's Medical College Hospital for their generous support to this study.

Financial support and sponsorship

Funded by the grants for internal faculty from the Institutions Research Society, St. John's Medical College Hospital, Bengaluru, India.

Ethical disclosures

Protection of human and animal subjects. This research do not used human nor animal material.

Right to privacy and informed consent. The authors declare that no data that enables identification of the patients appears in this article.

Conflict of interest. None declared

Footnotes

This study was partially presented at the 17th International Congress on Infectious Diseases (Abstract no. 41.081).

Author's contribution

Concept and design (SN, RM), data collection and laboratory work (BSK, SPC), data analysis, interpretation and drafting of the manuscript (BSK, SPC, SN), critical review of the manuscript (RY, RM). All authors read and approved the final version of the manuscript.

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