

# Molecular Epidemiology of Extensively-Drug Resistant *Acinetobacter baumannii* Sequence Type 2 Co-Harboring *bla*<sub>NDM</sub> and *bla*<sub>OXA</sub> From Clinical Origin

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**Background:** The therapeutic management of carbapenem-resistant *Acinetobacter baumannii* (CR-AB) represents a serious challenge to the public health sector because these pathogens are resistant to a wide range of antibiotics, resulting in limited treatment options. The present study was planned to investigate the clonal spread of CR-AB in a clinical setting.

**Methodology:** A total of 174 *A. baumannii* clinical isolates were collected from a tertiary care hospitals in Lahore, Pakistan. The isolates were confirmed by VITEK 2 compact system and molecular identification of *recA* and *bla*<sub>OXA-51</sub>. Antimicrobial profile and the screening of carbapenem-resistant genes were carried out using VITEK 2 system and PCR, respectively. The molecular typing of the isolates was performed according to the Pasteur scheme.

**Results:** Of the 174 *A. baumannii* isolates collected, the majority were isolated from sputum samples (46.5%) and in the intensive care unit (ICU, 75%). Among these, 113/174 (64.9%) were identified as CR-AB, and 49.5% and 24.7% harbored *bla*<sub>OXA-23</sub> and *bla*<sub>NDM-1</sub>, respectively. A total of 11 (9.7%) isolates co-harbored *bla*<sub>OXA-51</sub>, *bla*<sub>NDM-1</sub>, and *bla*<sub>OXA-23</sub>. Interestingly, 46.9% of the CR-AB belonged to sequence type 2 (ST2; CC1), whereas 15.9% belonged to ST1 (CC1). All of the CR-AB isolates showed extensive resistance to clinically relevant antibiotics, except colistin.

**Conclusion:** The study concluded CR-AB ST2 clone harboring *bla*<sub>OXA-23</sub> and *bla*<sub>NDM-1</sub> are widely distributed in Pakistan's clinical settings, which could result in increased mortality. Strict compliance with the National Action Plan on Antimicrobial Resistance is necessary to reduce the impacts of these strains.

**Keywords:** *Acinetobacter baumannii*, *bla*<sub>OXA</sub>, *bla*<sub>NDM</sub>, MIC, MLST

## Introduction

The development and spread of carbapenem-resistant *Acinetobacter baumannii* (CR-AB) represents a serious global public health dilemma.<sup>1</sup> Extensively drug resistant *A. baumannii* produce resistance to all or almost all medically approved antibiotics.<sup>2</sup> *A. baumannii* can cause severe infections, such as septicemia, urinary tract infections, wound infections, and ventilator-associated pneumonia, and *A. baumannii* has been associated with nosocomial infections.<sup>3</sup> *A. baumannii* has been designated as a global priority pathogen by the World Health Organization (WHO)<sup>4</sup> and is listed as an ESKAPE pathogen, which effectively resist to various antibacterial drugs, and restricting therapeutic options.<sup>5</sup> Every year, over 0.7

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million people die from infections due to antimicrobial-resistant (AMR) bacteria globally. By 2050, an individual is estimated to die from infection with an AMR pathogen every 3 seconds, and the global costs associated with AMR infections are expected to reach \$100 trillion.<sup>6</sup> The Centers for Disease Control and Prevention has reported that CR-AB is responsible for 700 mortality, and \$281 million are spent on the therapeutic management of CR-AB infections annually.<sup>7</sup> CR-AB develop resistance through numerous mechanisms, such as the production of  $\beta$ -lactam enzymes, porin proteins loss, and efflux pump overexpression.<sup>8</sup> Infections caused by CR-AB pathogens are difficult to treat due to the acquisition of various resistant genes such as class D oxacillinase (OXA) and metallo- $\beta$ -lactamase (MBL).<sup>9</sup> The New Delhi metallo- $\beta$ -lactamases (NDM) belong to a superfamily of class B MBL-producing bacteria,<sup>10</sup> which was first identified in a Swedish patient who sought medical attention in New Delhi, India, in 2009.<sup>11</sup> NDM pathogens are resistant to  $\beta$ -lactams and other antibiotic classes, including aminoglycosides and fluoroquinolones, and are only susceptible to the potentially toxic colistin and polymyxin B antibiotics.<sup>12</sup> However, resistance to colistin has also been reported at the global level.<sup>13</sup> The chimeric gene *bla*<sub>NDM-1</sub> is derived from the fusion of aminoglycoside phosphotransferase (*aphA6*) and an existing *bla*<sub>MBL</sub> gene,<sup>14</sup> and the promoter for ISAba125, which is encoded upstream, drives the expression of *bla*<sub>NDM-1</sub>. The genes *bla*<sub>OXA-23</sub>, *bla*<sub>OXA-40</sub>, and *bla*<sub>OXA-58</sub> encode the most frequently identified OXAs, whereas, *bla*<sub>OXA-51</sub> is intrinsic to resistant *A. baumannii* and can be used to identify the *Acinetobacter* spp.<sup>15</sup> These pathogens continue to represent critical issues in many health-care facilities worldwide, despite the implementation of infection control practices.<sup>16</sup> Currently, little evidence has supported the presence of CR-AB in Pakistan. However, a recent study documented the prevalence of NDM-1-producing *A. baumannii* at a tertiary care hospital in Lahore.<sup>11</sup> The aim of the present study was to determine the antimicrobial susceptibility testing, identify the most prevalent sequence types (STs), and investigation of resistant mechanism of CR-AB clinical isolates in Pakistan.

## Methodology

### Ethical Considerations

Before initiating this research study, ethical approval in accordance with the Declaration of Helsinki was obtained from the Ethical Review Committee, Government College University, Faisalabad. Additionally, prior to collecting the clinical samples, informed consent was obtained from each study participant. Informed consent was read to the person in the language they understand and signed appropriately. They were willing to provide a sample and utilize the isolates for the study. They were assured that the samples would be used solely for research purposes and that personal information would be kept confidential.

### Clinical Isolates

A convenience sample consisting of 174 non-duplicate *A. baumannii* clinical isolates were collected from a tertiary care hospital in Lahore between September 2020 to December 2020. Only a single strain was collected from each case irrespective of the infection site and preserved at  $-80^{\circ}\text{C}$  in Luria Bertani broth containing 16% glycerol. *A. baumannii* strains were primarily acquired from sputum (n=81), pus (n=42), blood (n=19), urine (n=18), throat swabs (n=7), cerebrospinal fluid (CSF) (n=3), nasal swab (n=2), seminal fluid (n=1), and high vaginal swab (n=1). The collection and transport of the clinical strains was done aseptically.

### Isolate Identification by VITEK 2 System

Isolates were sub-cultured on blood and MacConkey agar (Oxoid, UK), and the plates were incubated at  $37^{\circ}\text{C}$  overnight under aerobic conditions. Isolates were identified by Gram staining and culture morphology and were confirmed using Gram-negative identification (GN) cards in the VITEK 2 compact system (bioMérieux, France).

### Molecular Confirmation of *A. baumannii* Strains

*A. baumannii* were further confirmed by the detection of the intrinsic *bla*<sub>OXA-51</sub> and *recA* genes using specific primers, as described previously. The amplicons were subjected to gel electrophoresis, followed by examination under ultraviolet (UV) light using a gel documentation instrument (Bio-Rad, UK).

## Minimum Inhibitory Concentration (MIC)

An antibiogram was generated using various classes of antibiotics, such as mezlocillin, piperacillin, piperacillin/tazobactam, ampicillin/sulbactam, cefepime, cefotaxime, ceftazidime, imipenem, meropenem, gentamicin, amikacin, ciprofloxacin levofloxacin, tetracycline, tobramycin, trimethoprim/sulfamethoxazole, and colistin, in the VITEK 2 compact system. The MIC for colistin was determined using the microbroth dilution method. Interpretation of the antimicrobial susceptibility testing was carried out as per CLSI 2019 guidelines.<sup>17</sup>

## Detection of Carbapenemase and MBL Phenotypes

The modified Hodge test (MHT) was performed to detect carbapenemase, as described previously.<sup>18</sup> A 0.5 McFarland *Escherichia coli* ATCC 25922 turbidity standard was prepared and diluted 1:10 with sterile normal saline. The bacterial suspension was lawned on Mueller-Hinton agar (MHA) plates, and a meropenem (10 µg) disk was placed in the center. Isolates were streaked from the periphery of the disk to the edge of the plates. The MHT was considered positive if the isolates displayed clover leaf-like indentation.

MBL detection was performed using the double-disk synergy test (DDST), as described previously.<sup>19</sup> Briefly, a 0.5 McFarland bacterial suspension was lawned on MHA plates, and 2 meropenem and 2 ertapenem disks were placed at a distance of 25 mm, and 10 µL of 5 M ethylenediaminetetraacetic acid (EDTA) solution was added to one of each meropenem (10 µg) and ertapenem (10 µg) disk. MBL production was considered positive if the EDTA disks showed a zone of inhibition 5 mm larger than those for the non-EDTA disks.

## Detection of the *bla*<sub>NDM</sub> and *bla*<sub>OXA</sub> Genotypes

DNA from the clinical isolates was extracted using a commercially available bacterial genomic DNA kit (Thermo Scientific, UK). DNA purity was checked at 260 nm and 280 nm (NanoDrop Spectrophotometer, Thermo Scientific, UK). The DNA integrity was determined by electrophoresis (Bio-Rad, UK). These were following primers; *bla*<sub>NDM</sub>-F-5' ATGGAATTGCCCAATATTATGCAC-3' *bla*<sub>NDM</sub>-R-5' TCAGCGCAGCTTGTCGGC-3', NDMV-F-5' TGGC

TTTTGAAACTGTTCGCACC-3' NDMV-R-5' CTGTCA CATCGAAATCGCGCGA-3', *bla*<sub>OXA-23</sub>-like-F 5' GAT CGGATTGGAGAACCAGA-3' *bla*<sub>OXA-23</sub>-like-R-5' ATT TCTGACCGCATTTCCAT-3', *bla*<sub>OXA-24</sub>-like-F-5' GG TTAGTTGGCCCCCTTAAA-3' *bla*<sub>OXA-24</sub>-like-R-5' AGT TGAGCGAAAAGGGGATT-3', *bla*<sub>OXA-58</sub>-like-F-5' AAGTATTGGGGCTTGTGCTG-3', *bla*<sub>OXA-58</sub>-like-R-5' CCCCTCTGCGCTCTACATAC-3', *bla*<sub>OXA-51</sub>-like-F-5' TAATGCTTTGATCGGCCTTG-3' *bla*<sub>OXA-51</sub>-like-R-5' TGGATTGCACTTCATCTTGG-3', *bla*<sub>OXA-143</sub>-F-5' TGGCACTTTCAGCAGTTCCT-3' *bla*<sub>OXA-143</sub>-R-5' TAATCTTGAGGGGGCCAACC-3', and *recA*-F-5' ACAATGACATTGCAAGCAATTG-3' *recA*-R-5' CCAA TTTCATACGAATCTGG-3'. The presence of *bla*<sub>NDM</sub> was detected under the following conditions: initial denaturation at 95°C for 1 min, secondary denaturation at 95°C for 45 sec, annealing at 58°C for 45 sec, primary extension at 72°C for 1 min, and a final extension at 72°C for 5 min. To determine the specific genotypes, *bla*<sub>NDM</sub> was re-amplified using another set of primers for sequencing. Multiplex PCR was performed to sequence *bla*<sub>OXA-51</sub>, *bla*<sub>OXA-23</sub>, *bla*<sub>OXA-24</sub>, and *bla*<sub>OXA-143</sub> under the following conditions: initial denaturation at 94°C for 5 min, secondary denaturation at 95°C for 30 sec, annealing at 52°C for 40 sec, primary extension at 72°C for 50 sec, and a final extension at 72°C for 6 min.

## Multilocus Sequence Typing (MLST)

Multilocus sequence typing (MLST) was performed to determine the genetic diversity of carbapenem-resistant isolates according to the Pasteur scheme. The DNA sequences of 7 housekeeping genes (*cpn60*, *fusA*, *gltA*, *pyrG*, *recA*, *rplB*, and *rpoB*) were amplified, followed by purification and sequencing. The sequences were analyzed using the PubMLST database [https://pubmlst.org/bigdb?db=pubmlst\\_abaumannii\\_seqdef](https://pubmlst.org/bigdb?db=pubmlst_abaumannii_seqdef).

## Results

### Clinical Information of the Isolates

Of the 174 *A. baumannii* clinical isolates obtained in this study, the majority were recovered from male (64.9%) patients compared to female (35.1%) patients, with an age range from 1 year to 70 years (mean age: 35.8 years). Isolates were primarily recovered from sputum (n = 81; 46.5%), followed by pus (n = 42; 24%) and blood (n = 19; 10.9%) samples. There were 3 (1.7%) strains of *A. baumannii* isolates isolated from cerebrospinal fluid samples. A significant proportion of

**Table 1** Clinical Information for the Sources of *A. baumannii* Isolates (n = 174)

Clinical Information	n (%)
<b>Sex</b>	
Male	113 (64.9)
Female	61 (35)
<b>Age (years)</b>	
Range	1–70
Mean	35.8
<b>Clinical samples</b>	
Sputum	81 (46.5)
Pus swabs	42 (24.1)
Blood	19 (10.9)
Urine	18 (10.3)
Throat swabs	7 (4)
Cerebrospinal fluid	3 (1.7)
Nasal swabs	2 (1.1)
Seminal fluid	1 (0.6)
High vaginal swabs	1 (0.6)
<b>Hospital wards</b>	
Intensive care unit	131 (75.2)
Male surgical ward	13 (7.4)
Female medical ward	11 (6.3)
Operation theater	8 (4.6)
Male medical ward	6 (3.4)
Female surgical ward	5 (2.8)

the clinical samples (n = 131; 75.2%) were obtained from patients who were admitted to the intensive care unit (ICU) followed by the male surgical ward (MSW; n = 13; 7.4%) and the female medical ward (FMW; n = 11; 6.3%). The clinical information for the recovered samples is provided in Table 1.

## Antimicrobial Resistance Phenotypes

The antimicrobial susceptibility testing for 113 (64.9%) CR-AB isolates displayed 100% resistance to cephalosporins [cefepime ( $\geq 32$  µg/mL), ceftazidime ( $\geq 32$  µg/mL), cefotaxime ( $\geq 64$  µg/mL)],  $\beta$ -lactam inhibitors [piperacillin/tazobactam ( $\geq 128/4$  µg/mL), ampicillin/sulbactam ( $\geq 32/16$  µg/mL)], and carbapenems (imipenem and meropenem), with MIC breakpoints  $\geq 8$  µg/mL. In addition, 87.6% isolates were resistant to fluoroquinolones (ciprofloxacin), 75.2% to tetracycline (doxycycline), 81.4% to amikacin (aminoglycoside), and 75.2% to co-trimoxazole.

However, all of the strains were susceptible to colistin (Figure 1). The WHO AWaRe categorization assigns medically relevant antibiotics into Access (first- and second-line antibiotics), Watch (critical antibiotics), and Reserve (last-resort antibiotics) groups.<sup>20</sup> Interestingly, 76 *A. baumannii* isolates displayed resistance against 16 different AWaRe antibiotics, 78 were resistant to 15 antibiotics, 81 to 14 antibiotics, and 113 to 9 antibiotics (Figure 2).

## Phenotypic Detection of Carbapenemase and MBL

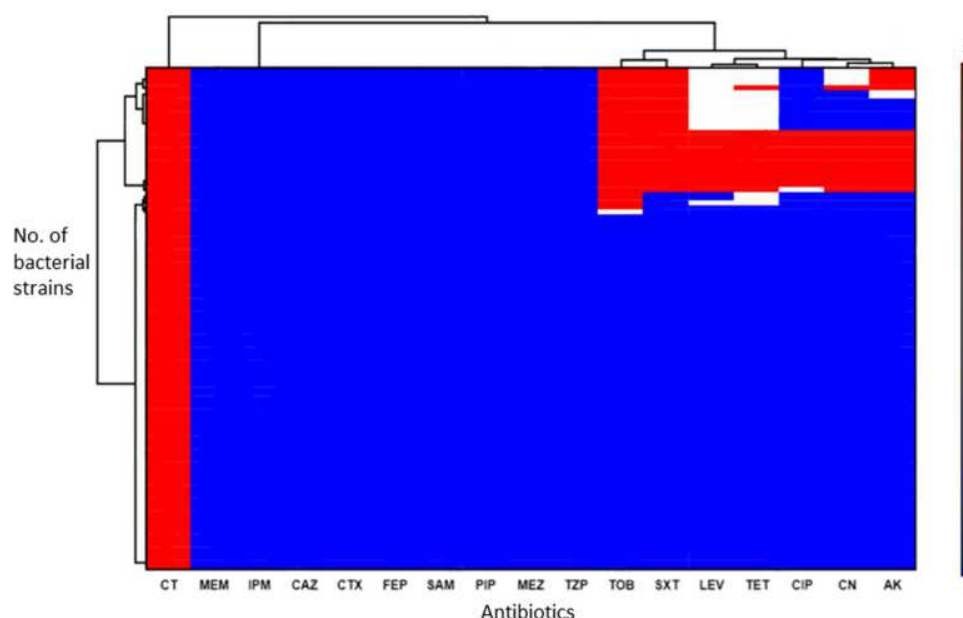
Of the 113 CR-AB isolates identified, 89 (78.7%) were positive for both carbapenemase and MBL production (Figure 3). A significant proportion (n = 64; 71.9%) of the MBL-producing CR-AB isolates were detected in ICU patients, followed by 14 (15.7%) in the MSW, 8 (9%) in the operation theater (OT), 2 (2.2%) in the male medical ward (MMW), 6 (6.7%) in the FMW, and 2 (2.2%) in the female surgical ward (FSW).

## Sequence Typing of Carbapenem Resistant *A. baumannii*

The MLST results for the 113 CR-AB clinical isolates revealed 7 different STs across the various samples and hospital wards (Table 2). A total of 53 (46.9%) CR-AB isolates belonged to ST2, which was the most predominant ST identified in this study, corresponding to clonal complex 2 (CC2), followed by 18 (15.9%) isolates that belonged to ST1 (CC1), 14 (12.3%) to ST589 (CC1), and 11 (9.7%) to ST7 (CC1). ST2 strains were primarily isolated from sputum samples (n = 27; 50.9%) and were more prevalent in patients admitted to the ICU (n = 21), whereas 13 (24.5%) ST2 isolates were detected in pus samples. Furthermore, 13 (72.3%) ST1 isolates were identified in sputum samples, most of which were found in the ICU (n = 12) cases. In addition, 5 (35.7%) ST589 isolates were also obtained from sputum collected from ICU cases.

## Genotypic Detection of Carbapenem-Resistant Genes

The CR-AB isolates (n = 113) were further screened for the presence of carbapenem-resistant genes. All CR-AB isolates harbored the intrinsic *bla*<sub>OXA-51</sub> gene, with a high prevalence of *bla*<sub>OXA-23</sub> (n = 56; 49.5%), followed by *bla*<sub>NDM-1</sub> (n = 28; 24.7%), *bla*<sub>OXA-58</sub> (n = 22;

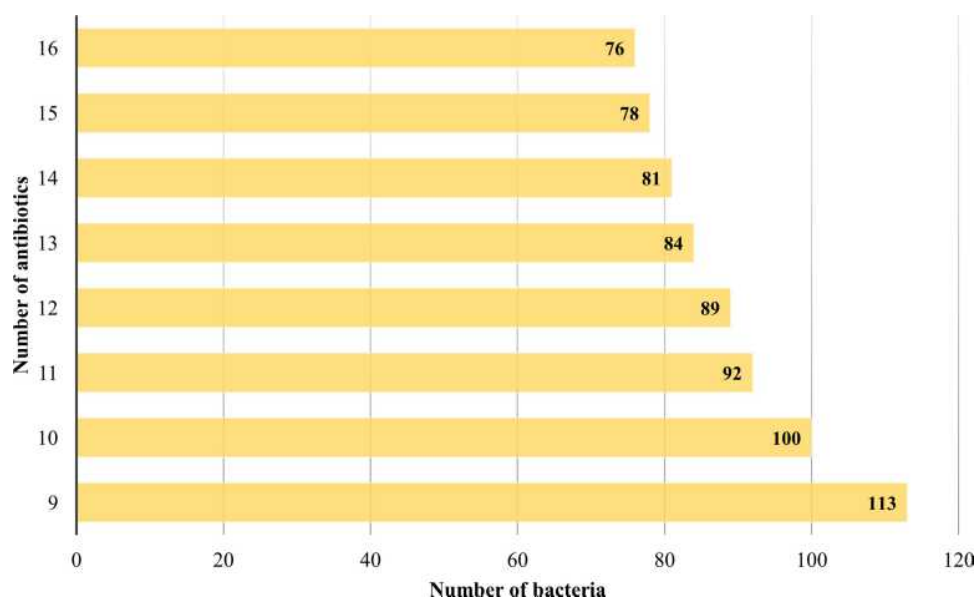


**Figure 1** The hierarchical clustering of carbapenem-resistant *Acinetobacter baumannii*. The antibiotics are shown on the x-axis, aligned with the bacterial strains on the y-axis. The scale on the y-axis shows bacterial resistance, intermediate resistance, and sensitivity from 1 to 3, respectively. The dendrogram shows the relationship among the different antibacterial drugs.

**Abbreviations:** CT, colistin; MEM, meropenem; IPM, imipenem; CAZ, ceftazidime; CTX, cefotaxime; FEP, cefepime; SAM, ampicillin/sulbactam; PIP, piperacillin; MEZ, mezlocillin; TZP, piperacillin/tazobactam; TOB, tobramycin; SXT, trimethoprim/sulfamethoxazole; LEV, levofloxacin; TET, tetracycline; CIP, ciprofloxacin; CN, gentamicin; AK, amikacin.

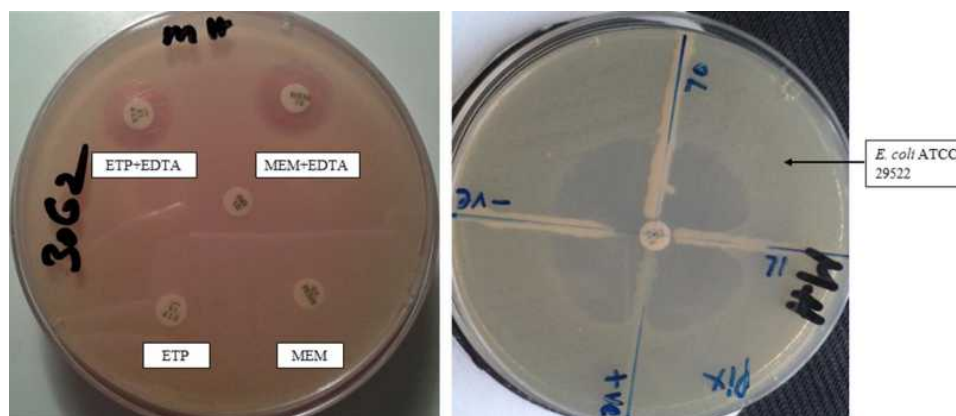
19.4%), and *bla*<sub>OXA-143</sub> (n = 3; 2.6%). In addition, 28 (50%) isolates harbored *bla*<sub>OXA-23</sub>, 14 (50%) harbored *bla*<sub>NDM-1</sub>, 12 (54.5%) harbored *bla*<sub>OXA-58</sub>, and 8 (80%) harbored *bla*<sub>OXA-24</sub>, all of which belonged to ST2. Interestingly, 11 (9.7%) isolates harbored *bla*<sub>OXA-51</sub>,

*bla*<sub>NDM-1</sub>, and *bla*<sub>OXA23</sub> together, 3 (2.6%) isolates harbored *bla*<sub>OXA-51</sub>, *bla*<sub>OXA-23</sub>, *bla*<sub>OXA-24</sub>, and *bla*<sub>NDM-1</sub>, and 7 (6.2%) harbored *bla*<sub>OXA-51</sub>, *bla*<sub>OXA-23</sub>, *bla*<sub>OXA-58</sub>, and *bla*<sub>NDM-1</sub>. In addition, 6 (5.3%) isolates harbored both *bla*<sub>OXA-51</sub> and *bla*<sub>OXA-23</sub>, and 17 (15%) harbored



**Figure 2** Carbapenem-resistant *A. baumannii* shows simultaneous resistance to multiple classes of antibiotics.





**Figure 3** Carbapenem-resistant phenotypes. The left photograph shows the DDST for MBL production, and the right photograph displays the MHT for carbapenemase production.

**Abbreviations:** ETP, ertapenem; MEM, meropenem; EDTA, ethylenediaminetetraacetic acid; MHT, modified Hodge's test; DDST, double disk susceptibility test; +ve, positive control; -ve, negative control; T-70, 71, tested organisms.

*bla*<sub>OXA-24</sub>, *bla*<sub>OXA-51</sub>, *bla*<sub>OXA-23</sub>, and *bla*<sub>OXA-58</sub> (Table 3).

## Discussion

CR-AB infections are difficult to treat and represent a persistent threat to Pakistan's fragile healthcare system.<sup>21</sup> These highly resistant pathogens are associated with extended hospital stays, increased mortality and morbidity, and added financial burden.<sup>22</sup> In this study, the intrinsic *bla*<sub>OXA-51</sub> was detected in all *A. baumannii* isolates, stressing the importance of using the *bla*<sub>OXA-51</sub> gene as an alternative marker for *Acinetobacter* spp., identification, as described previously.<sup>23,24</sup> *A. baumannii* is considered among the major nosocomial pathogens and has been identified in various areas of the hospital environment, including on medical devices and intravenous catheters.<sup>25</sup> In the present study, *A. baumannii* were recovered from numerous clinical specimens, primarily from sputum (46%) and pus (24%) samples, and were prevalent among patients admitted to the ICU (75%). Previously, multiple studies have indicated that the prevalence of *A. baumannii* in the ICU was becoming a serious nosocomial problem.<sup>26–28</sup> The CR-AB isolates in this study displayed resistance to all available  $\beta$ -lactam drugs, including carbapenems, which are considered to be the ultimate therapy for the infections produced by multidrug-resistant bacteria; although colistin remains the utmost effective drug, colistin is associated with considerable side effects. Among the identified isolates, 76 and 78 isolates showed simultaneous resistance to 16 and 15 medically relevant antibiotics, respectively, which have been categorized in the WHO AWaRe system. Previous data from Pakistan and other

parts of the world have documented a similar scenario, in which the primary methods used to treat infections are no longer able to fight these pathogens.<sup>11,21,29–31</sup> AMR is primarily caused by the acquisition of resistant determinants, which increase AMR phenotypes. The OXA-type- $\beta$ -lactamase is the most widely distributed class D enzyme found in gram-negative bacteria.<sup>32</sup> In this study, *bla*<sub>OXA-23</sub> was the predominant resistance determinant. OXAs are encoded by plasmid-mediated genes that can easily be transferred by conjugation. The main reservoir for the *bla*<sub>OXA-23</sub> gene exists in Asiatic countries, such as Pakistan, India, China, and Nepal. In addition, an extensive study conducted by the SENTRY Antimicrobial Surveillance Program in various Asian-Pacific counties also revealed the heavy presence of *bla*<sub>OXA-23</sub> in *A. baumannii*.<sup>33</sup> We also found *bla*<sub>OXA-24</sub>, *bla*<sub>OXA-58</sub>, and *bla*<sub>OXA-143</sub> genes, which had not been widely reported in Pakistan. The *A. baumannii* harboring *bla*<sub>OXA-58</sub> are primarily prevalent in China,<sup>34</sup> whereas *A. baumannii* harboring *bla*<sub>OXA-143</sub> are prevalent in Brazil<sup>35</sup> and Mexico.<sup>36</sup> The *bla*<sub>NDM-1</sub> gene encodes the most powerful class B MBL enzyme, which can hydrolyze a wide range of medically relevant antibiotics. The Indian subcontinent is considered to represent the primary reservoir for the emergence and spread of the *bla*<sub>NDM-1</sub> gene globally.<sup>6,11,37</sup> This study documented the prevalence of *bla*<sub>NDM-1</sub> in clinical *A. baumannii* isolates (24.7%) and found that this gene could be detected in isolates that simultaneously harbor the class D genes *bla*<sub>OXA-23</sub>, *bla*<sub>OXA-24</sub>, and *bla*<sub>OXA-58</sub>. Thus far, various studies have provided evidence to support the spread of *bla*<sub>NDM-1</sub> in Enterobacteriaceae in Pakistan rather than in non-fermenting bacteria.

**Table 2** Prevalence of Carbapenem-Resistant *Acinetobacter baumannii* Sequence Types in Different Clinical Wards

ST (n = 113)	CC	Allelic Profile	Clinical Infections and Hospital Wards							
			Sputum (n = 60)	Pus (n = 29)	Blood (n = 7)	Throat (n = 5)	Urine (n = 9)	CSF (n = 1)	Nasal (n = 1)	HVS (n = 1)
2 (n = 53, 46.9%)	2	2,2,2,2,2,2,2	27 (ICU:21, MMW:3, FMW:2, OT:1)	13 (ICU:7, MSW:3, FSW:1, OT:2)	4 (ICU:3, MMW: 1)	3 (ICU)	5 (FMW:2, ICU:3)	0	0	1 (FMW)
1 (n = 18, 15.9%)	1	1, 1, 1, 1, 5, 1, 1	13 (ICU:12, FMW:1)	3 (FSW:1, ICU:1, OT:1)	0	1 (ICU)	0	1 (OT)	0	0
589 (n = 14, 12.3%)	1	1,1,2,1,9,1,1	5 (ICU:3, FMW:1, OT:1)	4 (ICU:2, MSW:2)	1 (ICU)	1 (ICU)	2 (ICU)	0	1 (MSW)	0
7 (n = 11, 9.7%)	1	1,1,1,2,5,1,1	5 (ICU)	4 (MSW:2, FMW:1, ICU:1)	1 (ICU)	0	1 (ICU)	0	0	0
158 (n = 10, 8.8%)	158	41, 42, 13, 1, 5, 4, 14	6 (ICU)	2 (ICU:1, MSW:1)	1 (ICU)	0	1 (ICU)	0	0	0
23 (n = 5, 4.4%)	23	1, 3, 10, 1, 4, 4, 4	3 (ICU:2, FMW:1)	2 (ICU)	0	0	0	0	0	0
25 (n = 2, 1.7%)	25	3, 3, 2, 4, 7, 2, 4	1 (FMW)	1 (FSW)	0	0	0	0	0	0

**Note:** The ST profiles consist of 7 allele numbers, corresponding to *cpn60*, *fusA*, *gltA*, *pyrG*, *recA*, *rplB*, and *rpoB*, respectively (<https://pubmlst.org/abaumannii/>).

**Abbreviations:** ST, sequence type; CC, clonal complex; CSF, cerebral spinal fluid; HVS, high vaginal swab; ICU, intensive care unit; MMW, male medical ward; FMW, female medical ward; MSW, male surgical ward; FSW, female surgical ward; OT, operation theater.

However, recently published data have focused on the *bla*<sub>NDM-1</sub>-producing *A. baumannii* isolates in Pakistan.<sup>11,38</sup> The previously published data have also described the global detection of *bla*<sub>OXA-23</sub> and *bla*<sub>NDM-1</sub> genes in isolates that also harbor the *bla*<sub>OXA</sub> gene. The NDM gene can be readily transferred to *A. baumannii* via Tn125 by conjugation, and IS<sub>Aba125</sub> acts as a powerful promoter of the *bla*<sub>NDM-1</sub> gene.<sup>15,39,40</sup>

MLST is a promising technique for studying molecular epidemiology and investigating bacterial strain outbreaks in clinical and environmental settings. In this study, the *A.*

*baumannii* isolates belonged to 7 different STs: ST1, ST2, ST7, ST23, ST25, ST158, and ST589. Of these, the majority of the *A. baumannii* isolates (46.9%) belonged to ST2 (CC2). This clone was especially prevalent among critical care patients and was detected in isolate that also harbored the genes *bla*<sub>OXA-23</sub>, *bla*<sub>OXA-58</sub>, and *bla*<sub>NDM-1</sub>. The international clones, CC1 and CC2, were primarily associated with the enzymes *bla*<sub>OXA-23</sub>, *bla*<sub>OXA-24</sub>, and *bla*<sub>OXA-158</sub>. Mediterranean counties have documented the emergence of *A. baumannii* belonging to ST2, with fewer identified isolates belonging to ST1.<sup>41,42</sup> A Lebanon study reported the

**Table 3** The Co-Detection and Distribution of Carbapenem-Resistant Genes in Different CR-AB Alleles

ST of CR-AB (n = 113)	<i>bla</i> <sub>OXA-23</sub> (n = 56)	<i>bla</i> <sub>OXA-24</sub> (n = 10)	<i>bla</i> <sub>OXA-58</sub> (n = 22)	<i>bla</i> <sub>OXA-143</sub> (n = 3)	<i>bla</i> <sub>NDM-1</sub> (n = 28)
1 (n = 18)	6 (10.5%)	1 (10%)	2 (9%)	1 (33.4%)	2 (7.1%)
2 (n = 53)	28 (50%)	8 (80%)	12 (54.5%)	2 (66.6%)	14 (50%)
7 (n = 11)	4 (7.1%)	1 (10%)	2 (9%)	0	4 (14.2%)
23 (n = 5)	3 (5.3%)	0	2 (9%)	0	1 (3.5%)
25 (n = 2)	2 (3.57%)	0	0	0	1 (3.5%)
158 (n = 10)	4 (7.1%)	0	0	0	2 (7%)
589 (n = 14)	9 (16%)	0	4 (18%)	0	4 (14.2%)

**Abbreviations:** ST, sequence type; CR-AB, carbapenem-resistant *Acinetobacter baumannii*.

detection of ST2 *A. baumannii* harboring NDM-1.<sup>43</sup> Several studies from Pakistan have described the detection of *A. baumannii* ST2 clones.<sup>29,39</sup> ST2 (CC2) can survive in the clinical settings for longer times, allowing for the acquisition of AMR genes, which can complicate treatment. This report represents the first detection of the ST7 strain in Pakistan to date and need further comprehensive study to determine its diversification. This study was a compressive study examining the genes harbored by various CR-AB isolates that fills a gap by providing previously unavailable data regarding the coexistence of *bla*<sub>NDM-1</sub> and *bla*<sub>OXA</sub> genes in *A. baumannii* ST1, ST23, ST25, and ST158 isolates at the national level.

## Conclusion

This study highlights the significance of the intrinsic gene *bla*<sub>OXA-51</sub>, which can be used to rapidly detect *A. baumannii*. All strains were observed to be resistant to medically relevant antibacterials, with few treatment options available for infections associated with these isolates. Therefore, active monitoring and urgent management are necessary for clinical settings. The international clone ST2 was the most widespread isolates identified in public hospitals, simultaneously harboring *bla*<sub>OXA-23</sub>, *bla*<sub>OXA-24</sub>, *bla*<sub>OXA-58</sub>, and *bla*<sub>NDM-1</sub>, indicating that the circulation of these clones occurs at the international level, and this report represents the first reported detection of ST7 in Pakistan. Due to the impending threat associated with AMR bacteria, Pakistan's Ministry of Health has recently developed a National Action Plan (NAP) on Antimicrobial Resistance. Stakeholders must implement the NAP rapidly and effectively to halt the spread of these super-bacteria.

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## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

## Disclosure

All authors declared no conflicts of interest.

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