Molecular Detection of *bla*$_{oxa-23}$ Gene from Carbapenem Resistant *Acinetobacter Baumannii* Isolated from a Tertiary Care Hospital of Nepal

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Abstract

Background: Infections associated with *Acinetobacter baumannii* are increasing in many parts of the world especially the healthcare associated infections (HAIs). Antibiotics resistance is a great concern of public health which is either an inherent or adaptation property of microorganisms to resist the action of antibiotics. Carbapenem resistance, mainly among gram-negative bacteria is an ongoing problem that causes serious infections and dramatically limits the treatment alternatives. The prospective cross-sectional study was designed to detect the bla_{OXA-23} gene from carbapenem-resistant *A. baumannii* isolates in a tertiary care hospital of Nepal.

Methods: A total of 380 clinical specimens (tracheal aspirate, urine, sputum, blood and wound samples, Foleys tips, and catheter tips) were collected from the study population and were examined by microbiological procedures including Gram's staining, culture, and various biochemical tests. Antibiotic susceptibility testing (AST) was done as per the protocol of Kirby-Bauer disk diffusion technique and the CLSI guidelines while screening of carbapenemase production was checked through Modified Hodge Test (MHT) using Meropenem (10µg) disc. All the phenotypically positive results for carbapenemase production were further analysed by PCR and agarose gel electrophoresis for molecular detection of the bla_{OXA-23} gene.

Results: Among 380 specimens analysed, 55.3% (210/310) of samples were positive for bacterial growth where 15.7% (33/210) of bacterial isolates were *A. baumannii* and 69.7% (23/33) were carbapenem-resistant. High prevalence (21.2%) of *A. baumannii* was among the patients of age group 51-60 followed by 41-50 years (18.2%) years but the result was statistically insignificant (P>0.05) and more isolates were from ICU (60.6%) followed by post-operative patients (18.2%) The bla_{OXA-23} carbapenemase gene was found in 82.6% (19/23) of meropenem resistant isolates while 97% isolates of *A. baumannii* were susceptible to colistin.

Conclusion: The high rate of antibiotic resistance is funnelling the therapeutic options for the treatment of infections associated with *A. baumannii* which clearly shows a need for rational use of antibiotics. Systematic network surveillance should be established for monitoring and controlling the spread of the antibiotic-resistant gene of pathogenic bacteria especially in a resource-limited clinical setting like Nepal.

1. Introduction

The number of nosocomial infections caused by *Acinetobacter baumannii* is gradually increasing in recent years especially, more concern is in critically ill patients who are highly liable to infections and the risk factors for the infection are not well established [1]. The ubiquitous distribution, survival ability and high rate of resistance to the commonly used antibiotics are responsible for the emergence of *A. baumannii* as a significant nosocomial and opportunistic pathogen and have created a greater threat and challenge to treat the infections associated to it [2]. *A. baumannii* is considered as one of the frequently isolated opportunistic clinical pathogens and due to the tendency to acquire mechanism of antimicrobial

resistance it can cause community as well as healthcare-associated infections (HAIs) [3]. Since the early 1970s, multidrug-resistant (MDR) strains of *Acinetobacter* have been reported and *A. baumannii* is also associated with some of the disasters and conflict as a cause of osteomyelitis and wound infections like in 2002 Bali army operations, and earthquake of Turkey in 1999 [4]. The infections associated with *A. baumannii* range from pneumonia to meningitis but, more infections are reported from organs containing a high level of body fluids such as respiratory tract, peritoneal cavity, and urinary tract. In the majority of cases, it is believed that infections are developed after getting in contact with the hospital equipment contaminated by *A. baumannii* [5] which leads to severe illness with the high rate of patients death of about 30% [6]. Hospital-acquired infections like pneumonia, bloodstream and burn infections, meningitis, soft tissue infections, and osteomyelitis has made *A. baumannii* as a major potent and troublesome pathogen of health care setting and world health organization (WHO) has listed carbapenem-resistant *A. baumannii* as a priority pathogen of the 'Critical Group A' which needs new antibiotics to address the increasing antibiotic resistance (5, 7). Due to the unique ability to acquire or adapt antimicrobial resistance features *A. baumannii* is being a threatening pathogen to our antibiotic-based treatment strategy. Multiple number of genes are reported from *A. baumannii* which have role in attachment to abiotic surfaces and development of biofilm [8]. Multidrug resistant (MDR) strains have been isolated worldwide and it has been demonstrated that these strains can spread from area with high rates of antimicrobial resistance to other areas with historically low rates [9].

Basically, antibiotics of carbapenem group are considered as reserve drug for the treatment of multi-resistant *A. baumannii* caused infections but carbapenem-resistant strains of *A. baumannii* have been reported from multiple places [10]. *A. baumannii* can be resistant to carbapenem by various means but, mainly it is determined by hydrolysis of antibiotics by bacterial enzymes especially carbapenem-hydrolysing β-lactamases group of enzymes like oxacillinases which has six-subtypes which correspond to class D acquired $bla_{\text{OXA-23}}$-like (OXA-23, 27, and 49), chromosomal *bla*OXA-51-like, *bla*OXA-24/40-like (*bla*OXA-24-26, 40 and 72), *bla*OXA-58-like, *bla*OXA-235-like (OXA-235 to 237) and *bla*OXA-143-like enzymes and several antibiotic resistance cases due to production of acquired *bla*OXA have been identified in multiple places while *A. baumannii* carrying the $bla_{\text{OXA-23}}$-like gene is worldwide in distribution (11, 12, 13). Some of the insertion sequence (IS) elements like *ISAba1*, *ISAba2*, *ISAba3*, *ISAba4* and *IS18* have a positive role for the development of carbapenemases genes in *A. baumannii* and *ISAba1* can spread the carbapenemases genes among the species of *Acinetobacter* [14]. Wider distribution of antibiotic resistance $_{bla\text{OXA-23}}$-like gene has put the modern therapeutic options at a greater risk which has increased the hospital stay of patients with severe form of illness leading to economic and societal burden. Despite frequent antibiotic resistance cases being reported in Nepal there is very limited data on prevalence of $bla_{\text{OXA-23}}$-carrying *A. baumannii* so, this study was designed with an aim to determine the antibiotic susceptibility patterns and report the data on $bla_{\text{oxa-23}}$ gene among the carbapenem resistant *A. baumannii* isolates from a tertiary care hospital of central Nepal.

2. Materials And Methods

2.1 Study site and period

This cross sectional, hospital-based study was conducted at Annapurna Neurological Institute and Allied Science (ANIAS) Hospital and Annapurna Research Centre (ARC), Maitighar, Kathmandu, Nepal over a six-month period from February to August 2018.

2.2 Sample description

A total of 380 different clinical specimens including urine, blood, sputum, catheter tip, cerebrospinal fluid (CSF), tracheal aspirates, and central venous catheter (CVP) tips were collected from different wards of hospital. Specimens were from patients of general ward, post-operative ward and intensive care unit (ICU) of the hospital. A pre-formed questionnaire was used to record the patients' clinical and demographic data. Ethical approval for this study was given by the ethical review committee of National Health Research Council (NHRC), Ramshah Path, Kathmandu, Nepal (ref. no. 388/2018). Well-informed written consent was taken from all the enrolled patients in local language before each sample collection this article does not contain any individual patient data. For this study, age, sex, and antibiotic resistance were considered as independent variables while $bla_{\text{OXA-23}}$ gene was the dependent variable.

2.3 Collection and culture of samples

The specimens collected for this study were urine, blood, sputum, catheter tip, CSF, tracheal aspirates, and CVP tips. Each specimen was separately collected in a sterile container. All the specimens were processed and analysed in the microbiology laboratory of the hospital and PCR along with gel electrophoresis was performed in the ANIAS Research laboratory, Kathmandu, Nepal. Specimens like cerebrospinal fluid, urine, tracheal aspirates, and sputum were inoculated on the MacConkey agar (MA) and Blood agar (BA) plates then incubated at 37℃ for 24 hrs. After 24 hours of incubation at 37℃ plates were observed for the growth and further analysis. For all the blood specimens, blood in BHI (Brain Heart Infusion) broth was incubated aerobically for at least 7 days at 37℃. Each day one loopful of the inoculated broth was inoculated in MA and BA plates which were incubated at 37℃ overnight, growth on plates was observed after the incubation period and processed accordingly for the identification of bacterial strains. Other specimens like catheter tips, CVP tips, and Foley's tips were held with sperate sterile forceps and rolled over the surface of MA and BA plates then plates were incubated at 37℃ for overnight. After incubation, the growth of isolates was observed, and further identification tests were performed. All the inoculation and media preparation works were strictly performed under the laminar flow cabinet to prevent contamination.

2.4 Phenotypic identification of the bacterial isolates

After 24 hours of incubation, visual growth on the inoculated plates was observed and colony morphology was noted. The isolated colonies were then identified as per the morphology, gram staining and various biochemical tests which were catalase test, oxidase test, Voges Proskauer test, Methyl Red test (MR-VP), citrate test, urease test, oxidative-fermentative test, TSI (Triple Sugar Iron agar) test [15]. The identification of *Acinetobacter* spp. was done by standard laboratory procedure. After overnight

incubation, typical non-fermenting colonies of *Acinetobacter* spp. were identified. These colonies were then subjected to further processing via gram staining and other recommended biochemical tests [16]. *A. baumannii* was identified after performed series of biochemical tests such as positive catalase and citrate test, negative oxidase and urease test, non-motile, indole negative, oxidative in Hugh and Leifson's medium, negative gelatin hydrolysis test, acid production from glucose, lactose, xylose, galactose, mannose but not from sucrose and mannitol and ability to grow at both 37°C and 44°C, alkaline slant/alkaline butt i.e. glucose, lactose and sucrose non-fermenter, H_2S, and gas negative in TSI test [17].

2.5 Antibiotic susceptibility tests (AST) of *A. baumannii*

The AST test was performed as per the protocol of Kirby-Bauer disc diffusion technique to check the sensitivity and resistivity of *A. baumannii* isolates against the antibiotic discs. Identified strains of *A. baumannii* were inoculated on sperate Mueller–Hinton agar (MHA) plates and their susceptibilities to carbapenem were tested by disc diffusion method according to the guidelines of Clinical and Laboratory Standard Institutes (CLSI - M100-S25, 2015) [18]. Inoculum were prepared by suspending the single isolated colony on the nutrient broth and comparing the turbidity with 0.5 McFarland standards after proper incubation time. Carpet culture of bacterial suspension were performed on the MHA plate. After antibiotic discs were kept on the agar plate using sterile forceps then time was provided for the proper diffusion. Finally, plate was incubated for 18 hours at 37 °C. The diameter of zone of inhibition (ZoI) was measured for around all the discs then results were interpreted as recommended by CLSI guidelines and isolates were reported as 'resistant', 'intermediate' and 'sensitive' (Appendix- A). In this study, 12 antibiotic discs of different classes were used for antibiotic susceptibility test. We have used carbapenem class of antibiotics (Ertapenem-10 µg, Meropenem-10 µg, Imipenem-10 µg) to check the carbapenemase resistance among the *A. baumannii* isolates. As per the CLSI protocol, *A. baumannii* isolates which has shown susceptible or intermediate zones on AST for imipenem disc (16–21 mm) were further tested by Modified Hodge Test for phenotypic detection of carbapenemase production [19]. Antibiotics and quantity were selected based on prescription frequency by physician and availability at the time of study. Minimum inhibitory concentration (MIC) of the tested antibiotics were not determined due to unavailability of antibiotics powder and limited research fund.

2.6 Preservation of the *A. baumannii* isolates

After the AST, the confirmed carbapenem-resistant *A. baumannii* isolates in pure culture were preserved in 20% glycerol containing Tryptic Soya broth and kept at -70 °C until subsequent tests like Modified Hodge Test (MHT) and molecular tests.

2.7 Modified Hodge Test

It is a phenotypic screening test which is used to identify the carbapenemase producers. For the MHT, 0.5 McFarland dilution of the *Escherichia coli* ATCC 25922 in 5 ml of broth was prepared. Then it was diluted 1:10 by adding 0.5 ml of the 0.5 McFarland to 4.5 ml of saline. A lawn of the diluent was streaked on MHA and left to dry for 3–5 minutes. Then 10 µg meropenem/ertapenem antibiotic disc was placed at the centre of the plate. Then after, *A. baumannii* isolates were streaked straight from one edge of the disc

to the edge of the plate at 3 different places keeping the equal gap between them and plates were incubated for 24 hours at 35 °C in presence of ambient air. After the incubation period, clover leaf-type depression at the intersection of *E. coli* 25922 and *A. baumannii* was MHT positive while there was no growth of *E. coli* 25922 along the test isolates growth streak on the antibiotic disc diffusion area [20].

2.8 Molecular examination

2.8.1 Crude plasmid DNA extraction of *A. baumannii* and PCR reaction

Carbapenem resistant *A. baumannii* was preserved on Tryptic soya broth for plasmid DNA extraction process. DNA was extracted by alkaline hydrolysis method in which *A. baumannii* strain was cultured in LB (Luria Bertani) broth at 37 °C for overnight as describe previously [21]. The amount of extracted DNA was examined by spectroscopy at 260 nm. PCR reaction to identify bla_{OXA-23} gene was performed using specific primers (F: 5′-GATCGGATTGGAGAACCAGA-3′, B: 5′-ATTTCTGACCGCATTTCCAT-3′) ((Primers used reference: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4613023/) [22, 23]. The cycling conditions were performed with a preliminary denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 30 s, and annealing at 52 °C for 40 s, with a final extension at 72 °C for 10 minutes. PCR products were examined using 1% agarose gel electrophoresis containing 0.5 µg/ml ethidium bromide [11].

2.9 Quality Control

Mueller Hinton agar and the antibiotic discs were checked for their lot number, manufacture and expiry date, and proper storage. For the standardization of Kirby-Bauer test and for performance testing of antibiotics and MHA, control strains of *Escherichia coli* (ATCC 25922) were tested primarily. Quality of sensitivity test was monitored by maintaining the thickness of Mueller- Hinton agar at 4 mm and the pH at 7.2–7.4.

2.10 Statistical analysis

All the results were entered in the worksheet of Statistical Package of Social Sciences (SPSS 16.0). Chi-square test was used to determine the association of independent variables. A value of $\alpha \leq 0.05$ will be assumed wherever applicable and 95% confidence intervals along with the exact p-values will be presented.

3. Results

3.1 Bacterial growth

A total of 380 clinical specimens from in-patients (ICU ward, general ward, post-operative ward) were received and processed in the microbiological laboratory of ANIAS and Annapurna research centre during the study period. Among 380 analysed specimens, 55.3% (210/380) have shown aerobic bacterial growth

while 44.7% (170/380) specimens were negative for the bacterial growth. Out of 210 culture positive, 15.7% (33/210) isolates were confirmed as *A. baumannii* by microscopic observation of gram stain, series of biochemical tests, and growth temperature (Fig. 1).

3.2 Prevalence of *A. baumannii* in various clinical samples

The majority of identified *A. baumannii* strains (48.49%) were isolated from sputum specimens (16/33) followed by wound swab 24.3% (8/33), tracheal aspirates 12.1% (4/33), urine 9.1% (3/33), and catheters tips 3% (1/33) while no *A. baumannii* isolates were reported from CVP tips and CSF specimens (Table 1). Out of 33 isolates of *A. baumannii*, the most isolates were isolated from ICU (60.6%, 20/33), followed by post-operative ward (21.2%, 7/33) and general ward (18.8%, 6/33). (Fig. 2).

Table 1
A. baumannii isolates as per the analysed specimens

S.N.	Sample Type	Number of *A. baumannii* isolates	Percentage (%)
1.	Urine	3	9.1
2.	Sputum	16	48.5
3.	Blood	1	3.0
4.	CSF	0	0.00
5.	Tracheal Aspirates	4	12.1
6.	Catheters Tip	1	3.0
7.	Wound Swab	8	24.3
8.	CVP Tips	0	0.00
9.	**Total**	**33**	**100**

3.3 Gender and age wise distribution of *A. baumannii* isolates

Among 33 isolates of *A. baumannii*, the highest prevalence 57.6% was found in male patients (19/33) followed by female patients 42.4% (14/33). On the other hand, high prevalence 21.2% (7/33) of *A. baumannii* infection was reported in patients of age group of 51–60 years then was followed by patients of age group of 41–50 years (18.2%, 6/33) (Table 2).

Table 2
Gender and age wise distribution of *A. baumannii* isolates

	Gender of patients		
Age group (years)	**Male**	**Female**	**Total [n(%)]**
10–20	3	2	5 (15.2%)
21–30	4	1	5 (15.2%)
31–40	1	3	4 (12.1%)
41–50	5	1	6 (18.2%)
51–60	4	3	7 (21.2%)
61–70	2	2	4 (12.1%)
71–80	0	2	2 (6.0%)
Total	**19 (57.6%)**	**14 (42.4%)**	**33 (100%)**

3.4 Antibiotic susceptibility profile of *A. baumannii* against different classes of antibiotics

Twelve antibiotic discs were used for AST where 97% (32/33) of *A. baumannii* isolates were sensitive to colistin while only 12.1% (4/33) of isolates were sensitive to meropenem and imipenem antibiotics. High rate of resistance was seen against amikacin (100%), cephotaxime (97%), cotrimoxazole (97%), ampicillin (94%), amoxycillin-clavulanic acid (91%), piperacillin-tazobactam (91%), and ciprofloxacin (91%) (Table 3).

Table 3
Antibiotic susceptibility profile of *A. baumannii* against different classes of antibiotics

S.N.	Antibiotics	Concentration (µg)	RXN		
			R	I	S
			Number (%)	Number (%)	Number (%)
1.	AMP	10	31 (94)	0	2 (6.1)
2.	AK	30	33 (100)	0	0
3.	AMC	30 (20/10)	30 (91)	1 (3)	2 (6.1)
4.	CTX	30	32 (97)	1 (3)	0
5.	CIP	5	30 (91)	1 (3)	2 (6.1)
6.	COT	25 (23.75/1.25)	32 (97)	0	1 (3)
7.	C	30	29 (87.9)	2 (6.1)	2 (6.1)
8.	MRP	10	29 (87.9)	0	4 (12.1)
9.	IMP	10	29 (87.9)	0	4 (12.1)
10.	PIT	100/10	30 (91)	1 (3)	2 (6.1)
11.	CPM	30	27 (81.9)	3	3 (9.1)
12.	CL	10	1 (3)	0	32 (97)

Key: S = Sensitive, R = Resistant, RXN = Reaction, AMP = Ampicillin, AK = Amikacin, AMC = Amoxicillin/Clavulanic Acid, CTX = Cephotaxime, CIP = Ciprofloxacin, COT = trimethoprim + sulfamethoxazole (cotrimoxazole), C = Chloramphenicol, MRP = Meropenem, IMP = Imipenem, PIT = Piperacillin-Tazobactam, CPM = Cefepime, CL = Colistin (Methane Sulphonate)

3.5 Comparative evaluation of carbapenemase production by phenotypic tests

Two phonotypic tests AST and MHT were used to identify the carbapenemase producing *A. baumannii* where 87.9% (29/33) of isolates were screened positive for carbapenemase production by Kirby-Bauer disc diffusion where 69.8% (23/33) of isolates were confirmed as carbapenemase producer by Modified Hodge Test. Out of 29 carbapenem resistant *A. baumannii* by AST, 79.3% (23/29) of the isolates was found to be MHT positive while 20.7% (6/29) was found to be negative and the result was statistically significant (p = 0.001) (Table 4).

Table 4
Comparative evaluation of carbapenemase production by phenotypic tests

S. N.	Type of phenotypic test	Total no. of isolates	Carbapenemase producers {n(%)}		
i.	AST by Kirby-Bauer	33	29 (87.9)		
	MHT	33	23 (69.8)		
ii.		**Modified Hodge Test**			
iii.	Carbapenemase	Positive	Negative	Total	p-value
	R	23	6	29	**0.001**
	S	0	4	4	
	Total	**23**	**10**	**33**	

Key: S = Sensitive, R = Resistant, AST = Antibiotic Susceptibility Test, MHT = Modified Hodge Test

Table 5
Distribution of *bla*OXA-23 gene as per processed specimens and age of patients

S.N.	MHT	*bla*OXA-23 gene		Total	p-value
		Positive	**Negative**		
1.	Positive	19	4	23	0
	Negative	0	10	10	
	Total	**19**	**14**	**33**	
2.	**Age Group**	**Total no. of isolates**	bla**OXA-23 gene**		**p-value**
			Positive	**Negative**	
	10–20	5	1	4	0.657
	20–30	5	3	2	
	30–40	4	3	1	
	40–50	6	4	2	
	50–60	7	4	3	
	60–70	4	3	1	
	70–80	2	1	1	
	Total	**33**	**19**	**14**	
3.	**Type of specimens**	bla**OXA-23 gene**		**Total**	**p-value**
		Positive	**Negative**		
	Urine	2	1	3	0.310
	Sputum	9	7	16	
	Swab	4	4	8	
	Blood	0	1	1	
	Tracheal Aspirates	4	0	4	
	Foleys Tips	0	1	1	
	Total	**19**	**14**	**33**	

Key: MHT = Modified Hodge Test

3.6 Comparison of Modified Hodge Test with molecular detection of bla_{OXA-23} gene

Out of 23 MHT positive *A. baumannii*, 82.6% (19/23) of them harboured bla_{OXA-23} gene which was confirmed by conventional PCR and significant association was found between occurrence of bla_{OXA-23} gene and MHT ($p < 0.05$). High number (4/19, 21.1%) of *A. baumannii* isolated from patients of age group 40–50 and 50–60 years was found predominant for harbouring bla_{OXA-23} gene followed by 20–30, 30–40, and 60–70 years while least was confirmed from patients of age groups 10–20 and 70–80 years (5.3%, 1/19). There was no significant association between the age group of patients and harbouring of bla_{OXA-23} gene attribute by the pathogen ($p > 0.05$). Among analysed clinical specimens bla_{OXA-23} gene was found highest in sputum sample (47.4%, 9/19) followed by swab, tracheal aspirates (21.1%, 4/19) and urine (10.5%, 2/19) respectively. There was no statistically significant association between the specimens' type and harbouring bla_{OXA-23} gene attributes ($p > 0.05$) as presented in table 6 (**Table 6**).

Discussion

Emergence and spread of drug resistant *A. baumannii* have been recognized as a potent pathogen which has unique ability to survive in a hospital environment and remain for a long period of time and possess a great public health concern which is highly associated with HAIs [24]. Among 380 non-duplicate clinical specimens analysed, 210 (55.3%) were culture positive where 33 (15.7%) isolates were confirmed as *A. baumannii*. Identification and confirmation were done following gram staining, results of biochemical tests, and PCR detection of 16S rRNA and bla_{OXA-23} gene. Modified Hodge Test (MHT) was used as phenotypic test for the detection of carbapenemase enzyme among isolated *A. baumannii* which has shown 70% (23/33) isolates were positive for MHT test while AST has screened 87.9% (29/33) of isolates as carbapenem-resistant. PCR analysis has confirmed 57.6% (19/33) of isolates were positive for bla_{OXA-23} gene. In another study reported by Joshi et al. (2017) from the same hospital have reported 97.7% (43/44) carbapenem-resistant *A. baumannii* (CR-AB) where all the isolates (100%) were found positive for bla_{OXA-23} gene [22]. Similarly, Shrestha et al. (2015) have reported 49.6% (122/246) of MDR *A. baumannii* from a university hospital of Nepal where bla_{OXA-23} gene was observed in all isolates [25]. Earlier studies have also reported *A. baumannii* as a major cause of HAIs in different countries [26–28]. Huang et al. (2019) have also reported higher prevalence (94%) of bla_{OXA-23} gene from CRAB in China and Royer et al. (2018) have observed 78.3% of *A. baumannii* as MDR in Brazil where the associations between ISA*ba1*/OXA-51 and ISA*ba1*/OXA-23 was observed in 91.3% and 52.2% of the isolates, respectively [29, 30].

In our study, the highest number of *A. baumannii* isolates was found in sputum (48.5%) followed by wound swab (24.3%), tracheal aspirates (12.1%), urine (9.1%), blood (3%) and catheters tip (3%) respectively where the result was not statistically significant ($P = 0.310$). In agreement with this finding, Jaggi et al. (2012) have found maximum isolates from respiratory secretions (57.4%) followed by blood (23.8%) in India [31] and similarly highest isolates were reported in respiratory tract specimens in study conducted by Yadav et al. (2020) and Shrestha et al. (2016) from Nepal [32, 33]. This study highlights the higher prevalence of *A. baumannii* among ICU patients (60.6%) followed by post-operative (21.2%) and patients of general ward (18.2%) respectively. This result supports the role of *A. baumannii* as a major cause of ventilator-associated pneumonia (VAP) especially among critically ill patients and recent studies

shows the mortality rate of hospital patients from MDR-AB ranged from 52–66% and the high rate of MDR-AB transmission among ICU patients is very common mainly in resource limited setting [34]. Our study shows high prevalence of *A. baumannii* infections in patients of age group 51–60 years (21.2%) followed by 41–50 years (18.2%) and the result was statistically insignificant (P = 0.657) where more male patients (57.6%) were infected as compared to female (42.4%) but the result was not statistically significant (P = 0.657).

In this study, we have found high rate of resistance in amikacin (100%) and cephotaxime (97%) by disk diffusion technique which suggests amikacin and cephotaxime are no longer effective for the treatment of *A. baumannii* associated infections while 97% of isolates were sensitive towards colistin so, colistin could be the drug of choice for the treatment of *A. baumannii* caused infections in Nepalese patients. Meropenem and imipenem have shown similar rate (87.9%) of sensitivity in this study which is in agreement with the report reported in 2020 by Yadav et al. from a university hospital of Nepal. However, lower rate of amikacin resistance (86.3%) was reported by Yadav et al. (2020) where colistin has shown 100% sensitivity to the *A. baumannii* isolates [32]. Low rate of *A. baumannii* susceptibility towards the third and fourth classes of cephalosporin antibiotics is associated with the ESBL and AmpC β-lactamase positive strains. In essence, OXA-type carbapenemases are common in *A. baumannii* and the acquired bla_{OXA-23} gene is the main genetic element in Asian countries. The worldwide emergence of CRAB is a matter of concern to clinician which is mostly associated with grade D β-lactamases and MBLs. Plasmid carrying bla_{OXA-23} gene can be transported within the strains of *A. baumannii* via conjunction so, MDR and CRAB are speedily growing globally [35]. Other OXA-type genes like bla_{OXA-24} and bla_{OXA-58} are more common in isolates of *A. baumannii* from European region [36]. Other novel class D β-lactamases genes like $bla_{OXA-143}$ and $bla_{OXA-235}$ have been reported from different places of the USA, Mexico and Brazil [37, 38]. The CRAB is listed as a global priority pathogen in critical group by WHO which needs urgent efforts for the development of new antibiotics for the treatment of CRAB [7]. We have compared the two phenotypic tests; AST by Kirby-Bauer and MHT for the screening of carbapenemase producer *A. baumannii* where 87.9% (29/33) isolates were positive for carbapenemase production by Kirby-Bauer disk diffusion technique while 69.8% (23/33) isolates were carbapenemase producer by MHT and the result was statistically significant (P = 0.001). All the MHT positive isolates were assessed for the detection of bla_{OXA-23} gene using conventional PCR and all MHT positive isolates for carbapenemase production were positive for the target gene and the result was statistically significant ($P < 0.05$). Carbapenemase production was evaluated by different phenotypic techniques including MHT while the genotypic finding of carbapenemases among CRAB isolates using PCR is the most convincing [19]. Identification of carbapenemases among MDR strains of *A. baumannii* is crucial to plan the therapeutic regimen for clinician because CRAB limits the treatment options and can cause more deaths of infected patients. The production of carbapenem-hydrolysing class D β-lactamase (CHDLs) and MBL are the most common mechanisms which play positive role in carbapenem resistance [39]. The sensitivity of the MHT in our study (82.6%, 19/23) is in agreement with other studies reported previously where MHT has detected carbapenemase producer in 83.3%, and 73% among the screened isolates [40, 41]. As per CLSI 2018 guidelines, MHT is not recommend for carbapenemase detection as a phenotypic test which could be due

the poor specificity of the MHT when confirming some ESBL production happening with porin loss [42]. Nevertheless, 82.6% of MHT specificity in our study could be due to the detection of only one carbapenemase gene bla_{OXA-23} by the PCR.

This study highlights the high prevalence of bla_{oxa-23} gene among carbapenem resistant *A. baumannii* in Nepalese patients which suggests it is an urgent need to plan the control and treatment strategies for CRAB infected patients in our hospital setting otherwise it could reach a deadly shape. Hence, molecular based laboratory detection of resistant genes is a pressing need to prevent the dissemination of resistant strains.

Conclusion

The CRAB is considered a major challenge in the clinical setting of Nepal and cases are increasing in recent years. Emergence and spread of *A. baumannii* harbouring drug resistant genes like OXA-type carbapenemase, and NDM-1 will reduce the treatment options and cause a great public health problem in Nepal and beyond. Well-established mechanisms including timely detection of drug resistance genes by molecular techniques, surveillance of drug resistance patterns, control of surplus use of broad-spectrum antibiotics, and organized infection control strategies will be more necessitated to win the race against drug resistant *A. baumannii*. Systematic network surveillance should be established for monitoring and controlling the spread of the antibiotic-resistant gene of pathogenic bacteria especially in a resource-limited clinical setting like Nepal.

Limitations of the study

We cannot reveal the exact prevalence of CRAB harbouring bla_{oxa-23} gene without conducting nationwide study with large number of study population along with collection of data on possible risk factors. Due to time and fund limitation 16 s rRNA sequencing was not done in this study. So further studies in molecular level with long period should cover a greater number of participants from different parts of the nation is required generalize the result.

Abbreviations

ANAS Annapurna Neurological Institute and Allied Sciences

AST Antibiotic Susceptibility Testing

CDC Centre for Disease Control and Prevention

CRAB Carbapenem-resistant *Acinetobacter baumannii*

CSF Cerebrospinal Fluid

CVP Central Venous Pressure

HAIs Health Care Associated Infections

ICU Intensive Care Unit

IMP Imipenemases

MDR Multi Drug Resistant

MHT Modified Hodge Test

NDM New Delhi Metallo-β-Lactamase

OXA Oxacillinase Type

PCR Polymerase Chain Reaction

SSTI Skin and Soft Tissue Infection

UTI Urinary Tract Infection

VAP Ventilator Associated Pneumonia

VIM Verona integron-borne metallo-β-lactamase

WHO World Health Organization

Declarations

Ethics and consent to participate:

The ethical approval for this study was obtained from Nepal Health Research Council (NHRC), Nepal before the study. The issued letter of NHRC (Reg. No. 388/2018) can be presented on reasonable request. Written consent was taken from all the patients in local language before sample collection. This manuscript does not contain any individual human or animal data.

Consent for publication:

Not applicable

Availability of Data and Materials:

The datasets used and analysed during this study are available in excel sheets which can be obtained from the corresponding author on reasonable request.

Competing Interests:

The authors declare they do not have any competing interests.

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Author's Contributions:

LN is the primary author who designed the study methodology, performed laboratory investigations, and prepare the manuscript. AKS planned the experiments and supervised the project. BR helped in critical revision, data analysis and editing of the manuscript. AS contributed to the overall editing, review, and supervision of the project.

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Table

Table 6 not available with this version.

Figures

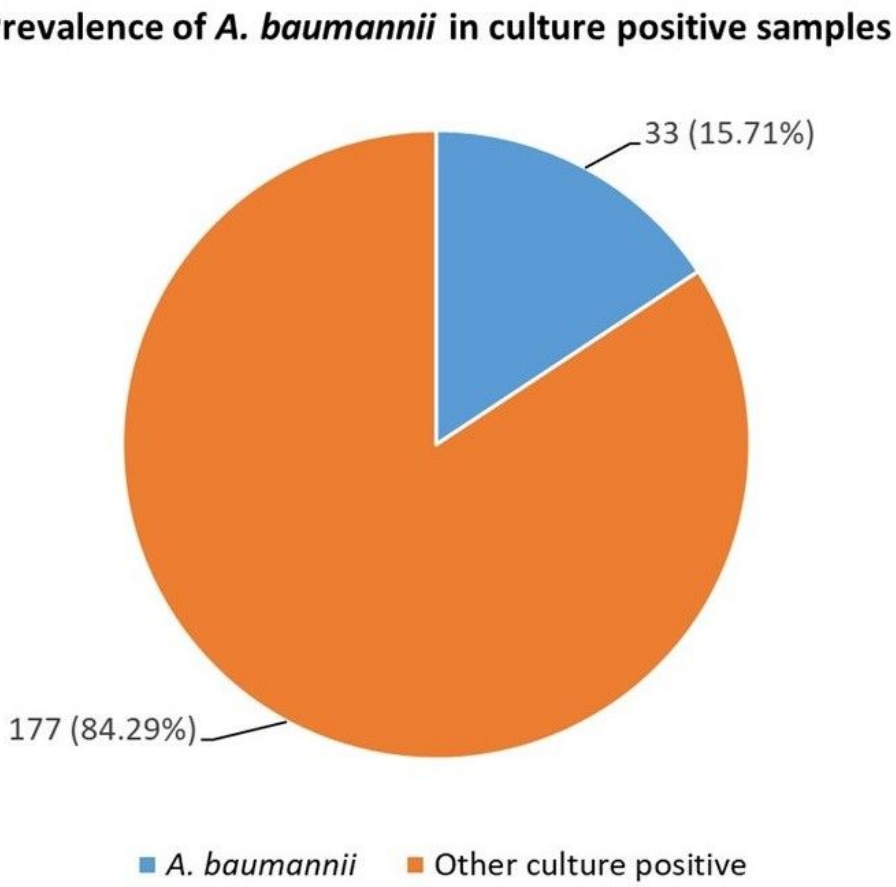


Figure 1

Prevalence of A. baumannii among culture positive bacteria

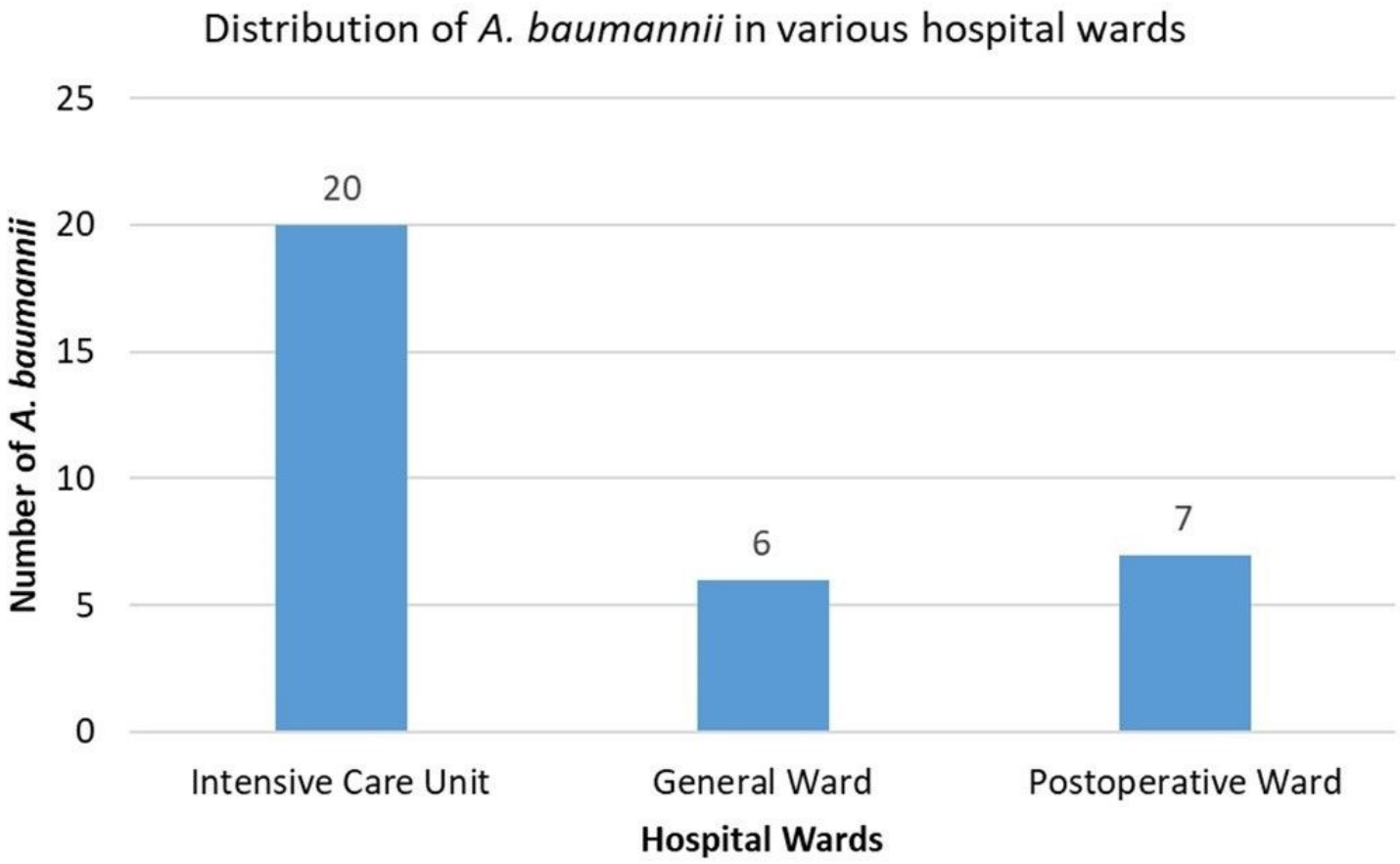


Figure 2

Prevalence of A. baumannii among different hospital wards

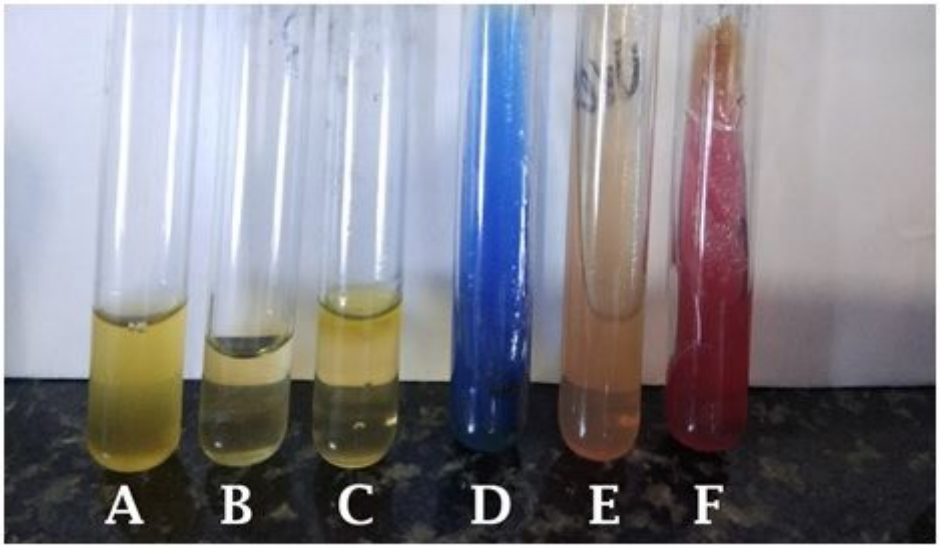
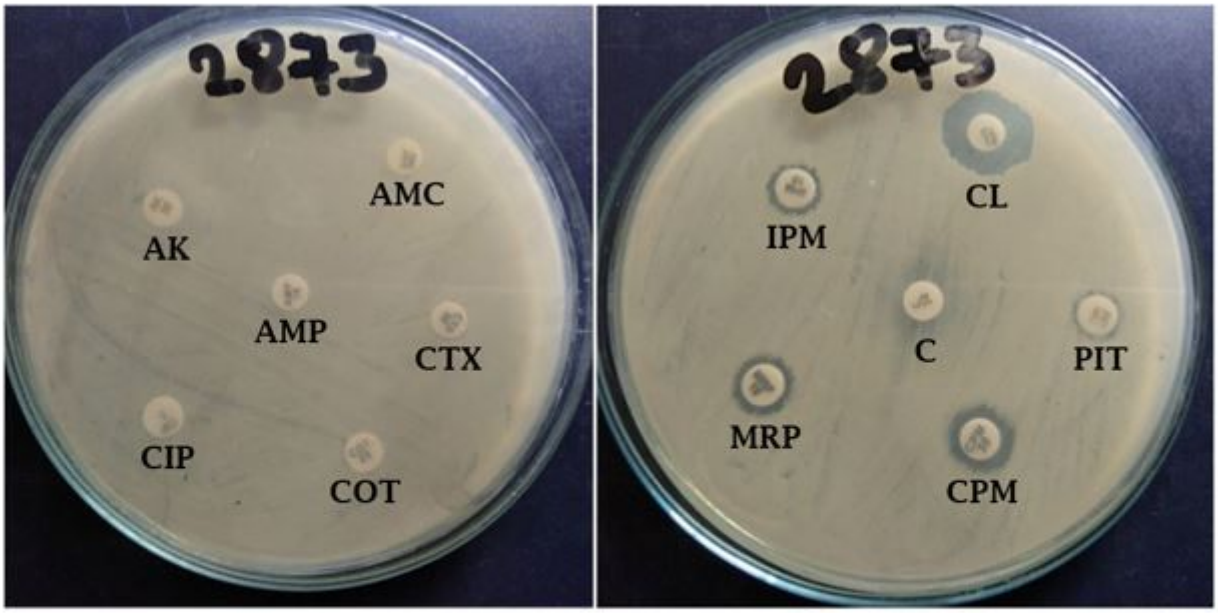


Figure 3

Results of biochemical tests of A. baumannii (Sample code: ST78) (A= MR -ve, B= VP -ve, C= SIM (H2S -ve, Indole -ve, Non-Motile), D= Citrate +ve, E= Urease -ve, F= TSI (Alk/Alk, H2S -ve, Gas -ve)) from left to

right



Resistant: AK, CIP, AMP, COT, CTX, AMC

Resistant: C, CPM, PIT, MRP, IPM
Sensitive: CL

Figure 4

Antibiotic susceptibility test of A. baumannii (Sample code: 2873)

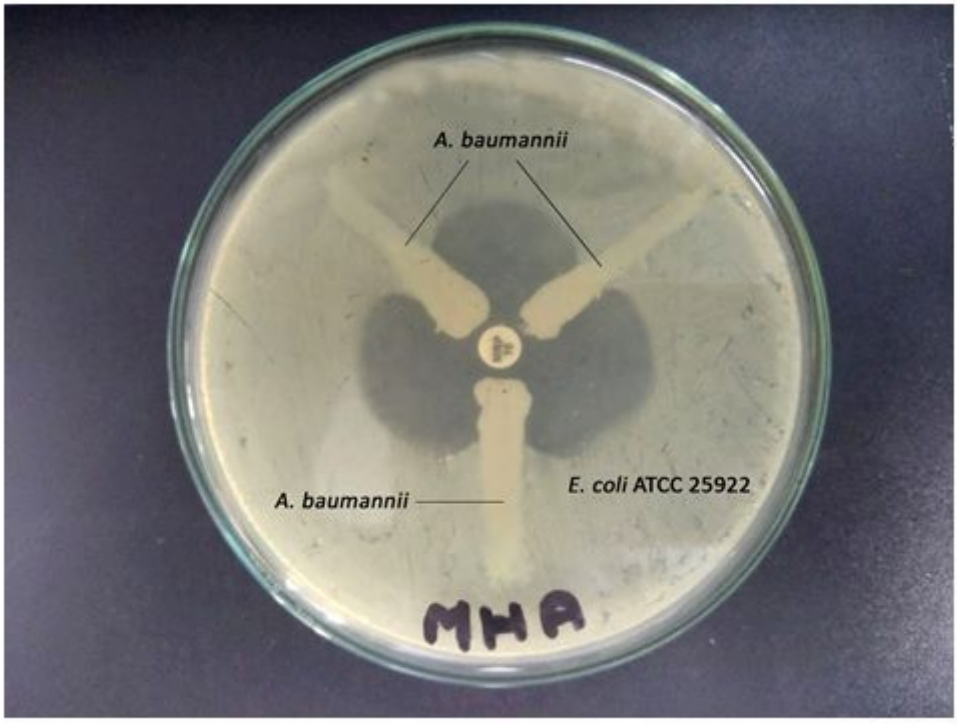


Figure 5

Positive Modified Hodge Test (MHT with Meropenem Disc, Sample code: 2873)

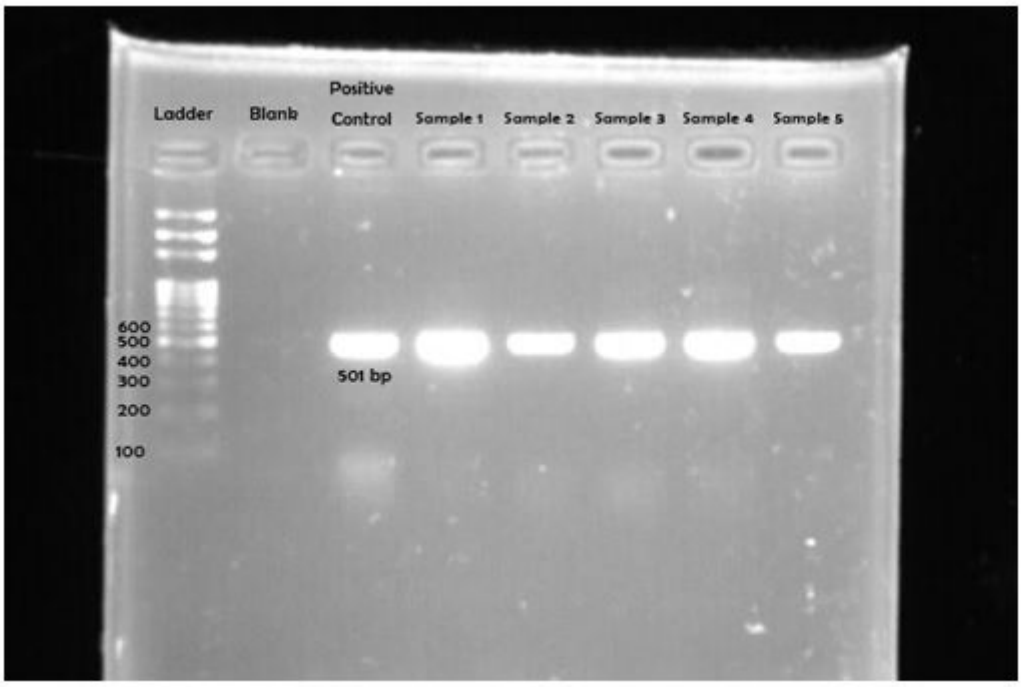


Figure 6

Gel Electrophoresis of PCR amplicons of blaOXA-23 gene (Sample 1 to 5)