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High antibiotic resistance and mortality with *Acinetobacter* species in a tertiary hospital, Nepal

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SETTING: Nepal Mediciti Hospital, Bhainsepati, Lalitpur, Nepal

OBJECTIVES: To determine antimicrobial resistance patterns, and the number and proportion of multidrug-resistant (MDR-) and extensively drug-resistant (XDR-) cases among all patients with *Acinetobacter* isolates between September 2018 and September 2019.

DESIGN: This was a hospital laboratory-based, cross-sectional study

RESULTS: *Acinetobacter* spp. (*n* = 364) were more common in respiratory (*n* = 172, 47.3%) and invasive samples such as blood, body fluids (*n* = 95, 26.1%). Sensitivity to AWaRe (Access, Watch and Reserve) Group antibiotics (tigecycline, polymyxin B, colistin) remained high. MDR (resistance to at least three classes of antimicrobial agents) (*n* = 110, 30.2%) and XDR (MDR plus carbapenem) (*n* = 87, 23.9%) isolates were most common in the Watch Group of antibiotics and found in respectively 99 (31.0%) and 78 (24.5%) patients (*n* = 319). Infected patients were more likely to be aged >40 years (*n* = 196, 61.4%) or inpatients (*n* = 191, 59.9%); 76 (23.8%) patients had an unfavourable outcome, including death (*n* = 59, 18.5%).

CONCLUSION: A significant proportion of MDR and XDR isolates was found; nearly one patient in five died. Robust hospital infection prevention and control measures (particularly for respiratory and invasive procedures) and routine surveillance are needed to reduce infections and decrease the mortality rate. Tigecycline, polymyxin B and colistin should be cautiously used only in MDR and XDR cases.

Acinetobacter genus was first isolated from soil in early 1914.[1] Although there are more than 50 species within the diverse *Acinetobacter* genus, *A. baumannii* is the most prevalent.[2] *Acinetobacter* spp. are aerobic, pleomorphic, non-motile, Gram-negative coccobacilli. They are abundant in nature and have been recovered from soil, water, animals, and in humans, where they are part of the normal flora of the skin and are frequently isolated from the throat and respiratory tract of hospitalised patients.[3] *Acinetobacter* infections are a growing global threat. The species are opportunistic pathogens, causing clinical conditions such as pneumonia, bacteraemia, urinary tract infections, wound infections, endocarditis and meningitis.[4] They are thus an important cause of healthcare-associated infection, especially in critical care settings.

From the late 1970s, there has been a rise in infection due to *Acinetobacter* spp., which emerged as a significant nosocomial pathogen, predominantly affecting critically ill, immune-compromised or ventilator-dependent patients. It is thus mainly associated with the increasing use of complex intensive care, including mechanical ventilation, central venous and urinary catheterisation and use of broad-spectrum antibiotics in hospitals.[5] It is sometimes referred to as "Iraqibacter" due to its seemingly sudden emergence in military treatment facilities during the 2003 Iraq War.[6,7] Multidrug-resistant (MDR) *Acinetobacter* spp. have spread to civilian hospitals, in part due to the transport of infected soldiers through multiple medical facilities.[8] Like many organisms, *Acinetobacter* spp. are becoming resistant to many drugs.

With the rise of widespread antimicrobial resistance, the WHO has advised the adoption of the AWaRe approach, which classifies antibiotics into three groups: Access, Watch and Reserve groups.[9] The Access group contains narrow-spectrum antibiotics recommended as first and second choice for most common infections. The Watch group contains broader spectrum antibiotic classes, indicated for specific use in a limited number of infections. These drugs are preferred over Access antibiotics and corresponding highest-priority agents on the list of critically important antimicrobial drugs.[10] The Reserve group consists of last-resort antibiotics reserved for treatment of confirmed or suspected infections due to MDR organisms.[11]

Acinetobacter spp. are resistant to several antibiotics, including priority Watch group antibiotics, such as cephalosporin, carbapenems; resistance to other reserve drugs such as colistin and polymyxins is also developing.[12] This rapid evolution toward MDR and extensively drug-resistant (XDR) isolates is alarming, and has become a health issue of national, as well as global, concern.[13]

An MDR *Acinetobacter* spp. is defined as an *Acinetobacter* isolate resistant to at least three classes of antimicrobial agents — all penicillins and cephalosporins, including inhibitor combinations, fluroquinolones and aminoglycosides.[14] An XDR *Acinetobacter* spp. is defined as an *Acinetobacter* isolate resistant to the three classes of antimicrobials in the MDR class, as well as to carbapenems.[15] The WHO has designated *Acinetobacter* spp. as a critical priority pathogen posing a great threat to human health, and for which new antibiotics are urgently needed.[16]

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KEY WORDS
multidrug resistant; MDR; extensively drug-resistant; XDR; adverse outcomes; operational research; Iraqibacter

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The prevalence of *Acinetobacter* spp. in Nepal has been reported to be around 11.8–12.5%,[17–19] with MDR levels between 72.4% and 95.2%.[18,20] Thus, there is a great threat of dissemination of resistant clones in the community, as well as in hospital settings. The burden of *Acinetobacter* spp. and the resulting clinical load in Nepal Mediciti Hospital, Lalitpur, a reputable tertiary centre in Nepal, is unknown. We determined the antimicrobial resistance patterns of *Acinetobacter* isolates, and prevalence of MDR and XDR isolates. We examined characteristics of patients infected with *Acinetobacter* spp.

METHODS

Study design

This was a hospital laboratory-based, cross-sectional study.

Setting

The study was conducted in Nepal Mediciti Hospital, Bhainsepati, Lalitpur, Nepal, a 700-bedded private tertiary healthcare centre located in the south-central part of Kathmandu valley. The hospital laboratory is the first Category A level laboratory recognised by the National Public Health Laboratory (NPHL), Kathmandu, Nepal, and has been accredited by the National Accreditation Board for Hospital and Healthcare System (NABH). The hospital has an active infection control committee.

Sample collection, processing and antimicrobial susceptibility testing

The hospital laboratory has an internal and external quality control programmes operated by the NPHL and NABH. Clinical samples from patients sent to the hospital laboratory were processed for aerobic bacterial culture on blood agar, MacConkey agar, chocolate agar and cystine lactose electrolyte deficient (CLED) agar, depending on the specimen type. All the media were incubated at 37°C for 24–72 hours. The suspected colonies were further processed for identification of *Acinetobacter* species using Gram staining, and conventional biochemical methods such as oxidase test, sugar fermentation, motility and indole production, citrate and urease consumption.[21] Their antimicrobial susceptibility tests were performed using the Kirby-Bauer disc diffusion method on Mueller Hinton agar medium and results were interpreted as per the Clinical and Laboratory Standards Institute (CLSI) guidelines.[22] Antibiotics were classified according to the WHO AWaRe groupings.[9]

Study population and duration

All isolates of *Acinetobacter* spp. from any biological sample sent for culture or sensitivity from any patient (in-, out-patient, emergency) from 1 September 2018 to 30 September 2019 were included.

Data collection

Demographic and biological sample characteristics of all patients with *Acinetobacter* spp. isolates were recorded from the Microbiology Laboratory registers and laboratory electronic records. Data included hospital identification (ID), age, sex, department, sample type, sample sent date, report issue date and hospital outcome. The dataset was counter checked by two independent microbiologists (MM and MC).

Data analysis and statistics

Data were analysed using Stata Statistical Software v15) (StataCorp, College Station, TX, USA; 2017). The rates of isolation of *Acinetobacter* spp. were presented as numbers and proportions. We assessed the prevalence of MDR and XDR using odds ratio (OR); the level of significance was set using $P < 0.05$. We used multiple logistic regression to explore the demographic and sample characteristics associated with identified drug resistance.

Ethics

Ethical approval for the study was taken from the National Health Research Council, Kathmandu, Nepal (ERB Protocol Registration No: 256/2020P) and the Ethics Advisory Group of the International Union against Tuberculosis and Lung Disease, Paris, France (EAG no. 02/20). As the study involved only the use of secondary data, no informed consent was necessary.

RESULTS

During the study period of 13 months from September 2018 to September 2019, there were 1,035 culture-positive specimens overall, of which a total of 364 were identified as *Acinetobacter* spp. (Table 1). The majority of the isolates came from respiratory samples, followed by invasive samples. The highest resistance was found to the Watch Group of antibiotics (Table 2), although there was also substantial resistance to the Access Group. Most samples were susceptible to the Reserve Group.

However, almost one third of the isolates were MDR, and one in four were also XDR (Table 3). Of the MDR isolates, risk factors identified in the multivariate analysis included the sample coming from an inpatient, or a surgical/wound swab (Table 3). The risks identified for XDR were inpatient sample, or a respiratory sample.

As 35 patients had multiple samples tested (n = 80), the total number of patients with *Acinetobacter* infection was 319 (Table 1). More isolates came from samples from male patients than female.

TABLE 1 Characteristics of samples (n = 364) and patients (n = 319) with *Acinetobacter* spp. attending Nepal Mediciti Hospital, Lalitpur, Nepal, September 2018–September 2019

	Acinetobacter-positive isolates	
Characteristics	*n*	%
Sample type (n = 364)		
Respiratory*	172	47.3
Invasive†	95	26.1
Urinary‡	59	16.2
Surgical/wound§	38	10.4
Age group, years (n = 319)		
⩽40	123	38.6
>40	196	61.4
Department (n = 319)		
Inpatients	191	59.9
Emergency	65	20.4
Outpatients	63	19.7
Hospital exit outcome (inpatient/emergency) (n = 246)		
Improved/discharged	170	69.1
Died	59	24.0
Discharged on request	11	4.5
Left against medical advice	4	1.6
Referred to other hospital	2	0.8

* Sputum, nasal/throat swab, bronchioalveolar lavage, suction tube.
† Blood, bone marrow, biopsy, body fluid, central venous line tip, cerebrospinal fluid.
‡ Urine, catheter tip.
§ Wound swab, pus.

TABLE 2 Antibiotic resistance pattern of *Acinetobacter* isolates (n = 364) among patients attending Nepal Mediciti Hospital, Lalitpur, Nepal, September 2018–September 2019

AWaRe Group	Total N	Resistant n	Resistant %*
Access			
Ampicillin	111	90	81.0
Amikacin	318	176	55.4
Gentamicin	289	150	51.9
Ampicillin/sulbactum	8	2	25.0
Watch			
Ceftriaxone	269	208	77.3
Cefotaxime	198	151	76.5
Ceftazidime	147	110	74.8
Cefepime	188	123	65.4
Ciprofloxacin	290	156	53.8
Levofloxacin	165	80	48.5
Pipracillin/tazobactum	328	152	46.3
Meropenum	333	151	45.4
Imipenum	334	138	41.3
Reserve			
Polymixin B	331	27	8.2
Colistin	326	27	8.3
Tigecycline	335	6	1.8

*Row percentage.
AWaRe = Aware, Watch and Reserve.

The highest number of isolates were from patients aged >40 years. More inpatients were infected by *Acinetobacter* spp. than emergency patients or outpatients. We found isolates from 99 (39%) patients showed an MDR pattern of drug resistance and 78 (24.5%) showed an XDR pattern (Table 3).

Among the infected individuals who were inpatients or emergency patients (n = 246), most recovered or were discharged (n = 170, 69.1%). However, nearly one in five patients died, and an adverse outcome was recorded in just under one in four (Table 1). There was no difference in antimicrobial resistance pattern between those who died or had any other adverse outcome: MDR strain (n = 110): 26 (23.6%) deaths (OR 1.4); 8 (7.3%) other adverse outcomes (OR 1.63); XDR strain (n = 87): 18 (20.7%) deaths; 5 (5.7%) other adverse outcome (OR 1.01).

DISCUSSION

We examined the antimicrobial resistance patterns of *Acinetobacter* spp. isolates among patients attending Nepal Mediciti Hospital from September 2018 to September 2019. About a third of the patients had MDR strains and a quarter XDR strains. Although most inpatients and emergency patients recovered or were discharged, adverse outcomes affected just under one fourth, and one in five died.

Perhaps not unexpectedly, most isolates were resistant to ampicillin, but nearly similar rate of resistant strains to the Watch group of antibiotics was observed; this was a matter of greater concern. Resistance to Access and Watch group antibiotics (β-lactams)[18] (gentamicin, carbapenems)[23] have previously been reported in Nepal. Carbapenems have been one of the most important therapeutic options for these infections,[13] but carbapenem-resistant strains are increasingly common,[24] although a recent report from India found low resistance to imipenem and meropenem.[25] Susceptibility to the Reserve antibiotics is not complete, and the rise of resistant strains to these antibiotics must be viewed with some concern.

MDR and XDR strains have previously been reported in Nepal[18,26] and North India,[27,28] although the prevalence of MDR and XDR strains in our study was lower. It is unlikely that this lower prevalence was simply due to improved infection prevention and control measures, as *Acinetobacter* spp. are largely nosocomial infections, transgressing infection control measures. Any isolation of *Acinetobacter* spp. should be viewed with concern.

In the MDR isolates, the most common factors we identified were hospital-based: samples coming from inpatients, and surgical/wound swabs. Similarly, the risks for XDR were inpatient, and respiratory samples, although the difference between sample sites

TABLE 3 Prevalence of multidrug-resistant and extensively drug-resistant isolates in samples and patients positive for *Acinetobacter* attending Nepal Mediciti Hospital, Lalitpur, Nepal, September 2018–September 2019

Characteristics	Total N	Multidrug-resistant isolates* n	Row %	OR	P value	aOR	95% CI	Extensively drug-resistant isolates† n	Row %	OR	P value	aOR	95% CI
Sample type	364	110	30.2					87	23.9				
Urinary‡	59	12	20.3	Ref		Ref		9	15.3	Ref		Ref	
Respiratory§	172	53	30.8	1.7	0.125	1.26	0.55–2.91	44	25.6	1.91	0.108	1.56	0.63–3.86
Surgical/wound¶	38	17	44.7	3.2	0.012	1.72	0.61–4.89	11	29.0	2.26	0.109	1.26	0.40–3.98
Invasive#	95	28	29.8	1.6	0.211	1.08	0.45–2.57	23	24.2	1.77	0.186	1.32	0.52–3.40
Patients	319												
Age ⩽40 years	123	34	27.6	Ref		Ref		25	20.3	Ref		Ref	
Age >40 years	196	65	33.2	1.3	0.300	1.38	0.79–2.41	53	27.0	1.45	0.176	1.56	0.86–2.83
Outpatients	63	4	6.4	Ref		Ref		4	6.4	Ref		Ref	
Inpatients	191	77	40.3	9.7	<0.001	9.73	3.35–28.36	62	32.5	7.09	<0.001	7.13	2.43–20.89
Emergency	65	18	27.7	5.65	0.003	5.00	1.55–16.16	12	18.5	3.34	0.047	2.73	0.81–9.18

* Defined as an *Acinetobacter* isolate resistant to at least three classes of antimicrobial agents – all penicillins and cephalosporins, including inhibitor combinations, fluroquinolones and aminoglycosides.[14]
† Defined as an *Acinetobacter* isolate resistant to the three classes of multidrug-resistant antimicrobials as well as to carbapenems.[15]
‡ Urine, catheter tip.
§ Sputum, nasal/throat swab, bronchioalveolar lavage, suction tube.
¶ Wound swab, pus.
# Blood, bone marrow, biopsy, body fluid, central venous line tip, cerebrospinal fluid.
OR = odds ratio; aOR = adjusted OR; CI = confidence interval; Ref = reference.

was not statistically significant. The presence of MDR and XDR *Acinetobacter* spp. in all types of samples and all groups of patients, but particularly in inpatients, is indicative of the high chance of the organism being found everywhere in the hospital environment, along with dissemination of resistant genes.

Inpatients are more likely to have MDR or XDR isolates than any other patient group. Outbreaks of *Acinetobacter* infection, including pneumonia, have occurred in healthcare facilities worldwide, including military treatment facilities caring for troops during the conflicts in Iraq and Afghanistan, hence the name Iraqibacter.[29] *Acinetobacter* is a challenging threat to hospitalised patients because it frequently contaminates healthcare facility surfaces and shared medical equipment.[30] The high rate of isolation of *Acinetobacter* spp. from respiratory samples in our study, as elsewhere,[31] is indicative of the risks of the generation of infectious aerosols from coughing and invasive procedures. *Acinetobacter* spp. present major challenges to physicians, as the cause of pneumonia, which becomes an easy source of further spread.

As elsewhere, patient characteristics such as age or sex made no difference to the isolation rate of MDR and XDR strains.[23,20] Nor was mortality or any other adverse outcome related to resistant strains.

While it was encouraging to note that a maximum number of *Acinetobacter* spp. isolates remain susceptible to the Reserve drugs (polymyxin B, colistin and tigecycline), there is a need to restrict use of these antibiotics to recorded failure of other antibiotics or authenticated MDR status of the isolate. Without such restriction, pan drug-resistant strains will quickly develop, with increase in deaths and the spread of resistance more widely.

Strength and limitations

This study explored the prevalence of *Acinetobacter* spp. infections in a tertiary healthcare centre located in the south-central part of Kathmandu valley over a 13-month period. The antibiotic resistance patterns were related to the WHO AWaRe grouping of antibiotics, focusing attention on relevant therapies. As patients from all over the Nepal and some parts of India are referred to the hospital, the study has implications outside the hospital.

The study was limited by the phenotypic detection of resistant strains of *Acinetobacter* spp. Genetic analysis of the resistant phenotype and drug resistance mechanism was not determined. Also, as full clinical information was not available, further exploration of the relevant patient characteristics, including whether the infection was contracted in the hospital, was not possible.

CONCLUSION

A significant proportion of isolates of *Acinetobacter* spp. were found to be MDR (30.2%) and XDR (23.9%) in a tertiary hospital in Nepal, with a substantial proportion of infected patients dying from their infection. Antibiotic susceptibility testing, aligned with the WHO AWaRe classification of drugs, is critical in the treatment of infections caused by *Acinetobacter* spp. This is particularly so in those with inadequate response to antibiotic therapy because of the increasing problem of antimicrobial resistance in *Acinetobacter* spp. Such a matter of concern, especially in inpatients with serious and complicated infections, needs urgent, hospital and country-wide action in line with the national action plan.

Furthermore, as some of the isolates are already showing some resistance to the Reserve Group of drugs, the development of new antibiotics is important. Ensuring robust infection prevention and control measures must be a priority in all healthcare settings, and in particular, in tertiary hospitals, to prevent the emergence of pan-resistant *Acinetobacter* spp.

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CONTEXTE : Hôpital de Mediciti, Bhainsepati, Lalitpur, Népal.
OBJECTIFS : Déterminer les profils de résistance antimicrobienne, le nombre et la proportion de cas multirésistants (MDR) et ultrarésistants (XDR) parmi tous les patients chez qui des isolats d'*Acinetobacter* ont été identifiés de septembre 2018 à septembre 2019.
MÉTHODE : Il s'agissait d'une étude transversale réalisée dans un laboratoire hospitalier.
RÉSULTATS : *Acinetobacter* spp. (*n*=364) étaient plus fréquentes dans les échantillons respiratoires (*n*=172, 47,3%) et invasifs comme le sang et les fluides corporels (*n*=95, 26,1%). La sensibilité aux antibiotiques de la classification AWaRe (« dont l'accessibilité est essentielle », « à utiliser sélectivement », « de réserve ») (tigécycline, polymyxine B, colistine) restait élevée. Les isolats MDR (résistance à au moins trois classes d'agents antimicrobiens) (*n*=110, 30,2%) et XDR (MDR plus carbapénème) (*n*=87, 23,9%) étaient plus fréquents dans le groupe des « antibiotiques à utiliser sélectivement » ; ils ont été observés chez respectivement 99 (31,0%) et 78 (24,5%) patients (*n*=319). Les patients infectés étaient plus susceptibles d'être âgés > 40 ans (*n*=196, 61,4%) ou hospitalisés (*n*=191, 59,9%). Un résultat défavorable a été observé chez 76 (23,8%) patients, dont des décès (*n*=59, 18,5%).
CONCLUSION : Une proportion significative d'isolats MDR et XDR a été observée ; près de un patient sur cinq est décédé. Des mesures concrètes de prévention et de contrôle des infections à l'hôpital (notamment pour les procédures invasives et respiratoires) et de surveillance de routine sont nécessaires pour réduire les infections et diminuer le taux de mortalité. La tigécycline, la polymyxine B et la colistine doivent être utilisées avec prudence, uniquement en cas de MDR et XDR.

Public Health Action (PHA) welcomes the submission of articles on all aspects of operational research, including quality improvements, cost-benefit analysis, ethics, equity, access to services and capacity building, with a focus on relevant areas of public health (e.g. infection control, nutrition, TB, HIV, vaccines, smoking, COVID-19, microbial resistance, outbreaks etc).