

## ***Pseudomonas aeruginosa* in Nepali hospitals: poor outcomes amid 10 years of increasing antimicrobial resistance**

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## **SUMMARY**

**OBJECTIVE:** To determine antimicrobial resistance patterns and prevalence of multi- (MDR, i.e., resistant to  $\geq 3$  classes of antimicrobial agents) and extensively (XDR, i.e., resistant to  $\geq 3$ , susceptible to  $\leq 2$  groups of antibiotics) drug-resistant strains of *Pseudomonas aeruginosa*.

**METHODS:** This was a cross-sectional study conducted in Nepal Medicity Hospital, Lalitpur, Nepal, using standard microbiological methods with Kirby Bauer disc diffusion to identify antimicrobial susceptibility.

**RESULTS:** *P. aeruginosa* ( $n = 447$ ) were most frequently isolated in respiratory ( $n = 203$ , 45.4%) and urinary samples ( $n = 120$ , 26.8%). AWaRe Access antibiotics showed 25–30% resistance, Watch antibiotics 30–55%. Susceptibility to AWaRe Reserve antibiotics remains high; however, 32.8% were resistant to aztreonam. Overall, 190 (42.5%) were MDR and 99 (22.1%) XDR (first Nepali report) based on mainly non-respiratory samples. The majority of infected patients were  $>40$  years ( $n = 229$ , 63.2%) or inpatients ( $n = 181$ , 50.0%); 36 (15.2%) had an unfavourable outcome, including death ( $n = 25$ , 10.5%). Our larger study showed a failure of improvement over eight previous studies covering 10 years.

**CONCLUSION:** Antibiotic resistance in *P. aeruginosa* occurred to all 19 AWaRe group antibiotics tested. Vulnerable patients are at significant risk from such resistant strains, with a high death rate. Sustainable and acceptable antibiotic surveillance and control are urgently needed across Nepal, as antimicrobial resistance has deteriorated over the last decade.

**KEY WORDS:** multidrug-resistant; extensively drug-resistant; adverse outcomes; operational research; infection control; MDR; XDR

*Pseudomonas aeruginosa* is a ubiquitous environmental bacterium with minimal nutritional requirements for survival and a remarkable ability to adapt to environmental challenges.<sup>1</sup> It is therefore an important pathogen, which has long been included in the Latin American Network for Antimicrobial Resistance Surveillance,<sup>2,3</sup> now covering North and South America.

*P. aeruginosa* persists in both community and hospital settings. In the hospital, it can be found in respiratory therapy equipment, antiseptics, soap, sinks, mops, medicines, and physiotherapy and hydrotherapy pools, while community reservoirs include swimming pools, whirlpools, hot tubs, contact lens solution, home humidifiers, soil and rhizosphere, and vegetables.<sup>4</sup> However, it requires reduced immunity to infect its host, as it is seldom a member of the normal microbial flora in humans.

Data covering 1986–2003 from the USA National Nosocomial Infections Surveillance system showed *P. aeruginosa* was the second most common cause of pneumonia (18.1%), the third most common cause of urinary tract infections (16.3%) and the eighth most frequently isolated pathogen from the bloodstream (3.4%).<sup>5</sup> It is one of the most common pathogens causing human opportunistic infections,<sup>6</sup> and a leading cause of healthcare-associated infection, especially in patients admitted to critical care units, as well as in patients undergoing surgery.<sup>7,8</sup> Colonisation rates may exceed 50% during hospitalisation.<sup>4</sup>

*P. aeruginosa* has become intrinsically resistant to several antibiotics due to the low permeability of the outer membrane, mutation in genes encoding porins, efflux pumps, penicillin-binding proteins and constitutive expression of  $\beta$ -lactamase, all contributing to resistance to  $\beta$ -lactams, carbapenems, aminoglycosides and fluoroquinolones.<sup>9</sup> Such resistance is becoming an increasingly important global health and economic problem,<sup>10,11</sup> although there has been some reported decrease in the prevalence of multidrug-resistant (MDR) organisms.<sup>12</sup>

MDR strains are now seen in hospitalised patients,<sup>13</sup> resulting in increased morbidity and mortality in infected and often vulnerable patients. One five-country study of nosocomial pneumonia noted a 30% prevalence of MDR *P. aeruginosa*.<sup>14</sup> The distribution of antimicrobial resistance (AMR) in *P. aeruginosa* shows distinct geographical patterning, with clonal strains transmitted from patient to patient.<sup>15–17</sup>

In 2011, the European Centre for Disease Prevention and Control and the US Centers for Disease Control and Prevention collaborated to standardise the definitions of *P. aeruginosa* and other organisms linked to healthcare-associated infections. Resistance to more than one antimicrobial agent in  $<3$  antimicrobial categories is defined as drug-resistant (DR) *P. aeruginosa*. The multidrug-resistant (MDR) phenotype is defined as *P. aeruginosa* resistant to  $\geq 3$  groups of antimicrobial agents. Extensively drug-resistant (XDR) bacteria are those

organisms resistant to  $\geq 3$  and susceptible to  $\leq 2$  groups of antibiotics, while pan drug-resistant (PDR) strains are resistant to all antibiotics.<sup>8,18</sup>

The prevalence of *P. aeruginosa* in Nepal was variously reported to be between 9.4%<sup>19</sup> and 17.1%.<sup>20</sup> As *P. aeruginosa* is frequently associated with healthcare-associated infections, especially in critical care areas, there is a great threat of dissemination of such resistant strains in the local community, as well as in hospital settings.

As a result, it is of crucial importance to isolate and identify the offending strain in order for appropriate antimicrobial therapy to be initiated. The objective of the present study was to determine the characteristics and patterns of AMR among isolates of *P. aeruginosa* recovered from clinical specimens in Nepal Medicity Hospital, Bhainsepati, Lalitpur, Nepal.

## **METHODS**

### *Study design*

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

### *Setting*

The study was conducted at the Nepal Medicity Hospital, a 700-bed, private, tertiary healthcare centre located in the south-central part of the Kathmandu valley. The hospital laboratory is the first Category A level laboratory recognised by the National Public Health Laboratory, Kathmandu, Nepal and has been accredited by the National Accreditation Board for Hospital and Healthcare System.

### *Study population and duration*

All isolates of *P. aeruginosa* from any biological sample sent for culture or susceptibility from any patient (in-, out-patient, emergency) from 1 September 2018 to 30 September 2019 were included. Demographic and outcome data on each patient were collected.

### *Sample collection, processing and antimicrobial susceptibility testing*

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 specimen type. All the media were incubated at 37°C for 24–72 h. *P. aeruginosa* were identified on the basis of colony morphology, pigmentation of colony, Gram staining, conventional biochemical methods and oxidase tests. The antimicrobial susceptibility tests were performed on Mueller-Hinton agar plates with commercially available

antibiotic discs (Hi-Media, Mumbai, India) using the Kirby Bauer disc diffusion method, and results were interpreted following standard Clinical and Laboratory Standard Institute (CLSI; Wayne, PA, USA) guidelines.<sup>21</sup> In the case of multiple samples from a patient, we selected the isolate showing the most resistance to antibiotics. Antibiotics were classified by the WHO AWaRe (Access, Watch and Reserve) groupings.<sup>22</sup>

### *Data collection*

Demographic and biological sample characteristics of all patients with *P. aeruginosa* isolates were recorded from microbiology laboratory registers and laboratory electronic records. Data included the hospital identification number, age, sex, department, sample type, sample sent date, report issue date, hospital outcome. The dataset was counter-checked by two independent microbiologists. We classified the AMR results published in previous studies from Nepal since 2010 into the AWaRe groups of antibiotics,<sup>22</sup> and compared the findings.

### *Data analysis and statistics*

Data were analysed using Stata software v15) (Stata Statistical Software: Release 15; StataCorp, College Station, TX, USA; 2017). The rates of isolation of *P. aeruginosa* were presented as numbers and proportions. We assessed the prevalence of MDR and XDR using odds ratios (ORs); the level of significance was set at  $P < 0.05$ . We used multiple logistic regression to explore the demographic and sample characteristics associated with identified drug resistance.

### *Ethics*

Ethical approval for the study was obtained from the National Health Research Council, Kathmandu, Nepal (ERB Protocol Registration No: 476/2020P) and the Ethics Advisory Group of the International Union Against Tuberculosis and Lung Disease (The Union), Paris, France (EAG no. 35/2020). As the study involved the use of secondary data only, no informed consent was necessary.

## **RESULTS**

We identified 447 isolates of *P. aeruginosa* from September 2018 to September 2019 from a variety of clinical samples from 362 patients (Table 1). Of these patients, 56 (15.5%) had multiple samples tested ( $n = 141$ ). The majority of the positive patients were over 40 years of age, inpatients or male. Although most patients recovered, 10.6% ( $n = 25$ ) died.

Three quarters of the 447 isolates were obtained from respiratory and urinary specimens (Table 1). The isolates were largely susceptible to the Access antibiotics, while the Watch Group of antibiotics had the highest proportion of resistant organisms, although there was also substantial resistance in some Reserve Group antibiotics (Table 2). Although some antibiotics were tested against a small number of strains, none of the 19 antibiotics tested showed complete susceptibility: the two in the Access group showed 26% and 29% resistance; in the 14 Watch group antibiotics, resistance ranged from 24% to 93%; resistance to the three Reserve group antibiotics varied from 6% to 33%.

Of the 447 *Pseudomonas* isolates, 190 (42.5%) were MDR and 99 (22.1%) were XDR. Respiratory isolates were significantly less likely to be MDR or XDR than the other sample sites (adjusted OR [aOR] 0.29, 95% confidence interval [CI] 0.15–0.58). Isolates from inpatients were significantly more likely to be MDR or XDR than those from outpatients or emergency patients (aOR 2.63, 95% CI 1.35–5.12) (Table 3). There was no difference in resistance between the younger and older patients. Similarly, there was no statistical difference between the reference group (with favourable outcome) and the 17 patients with an unfavourable outcome who also had MDR isolates (47.2%; aOR 1.16;  $P = 0.679$ ), or the nine with XDR isolates (25.0%; aOR 1.18;  $P = 0.692$ ).

We found eight previous studies in Nepal with sampling undertaken between 2010 and 2018 and sufficient information to compare with our study<sup>19,20,23–28</sup> (Table 4). The studies ranged from 6 months to 3 years, but all had smaller sample sizes than ours; five were undertaken in tertiary hospitals in Kathmandu,<sup>19,20,23,24,27</sup> two in Pokhara<sup>25,28</sup> and one in Bharatpur.<sup>26</sup> After adjustment to the equivalent of a 12-month study, only one study had a larger relative sample size.<sup>26</sup> The number of antibiotics tested ranged from eight to 14 (including non-AWaRe antibiotics), compared with our 19.

Of the eight studies, four<sup>20,24,26,27</sup> (sampled from 2012 to 2017) reported *P. aeruginosa* susceptible to at least one tested antibiotic; in our larger study, there were no antibiotics showing complete susceptibility. Reported MDR prevalence varied from 21% to 89%, but no study reported XDR isolates.

## DISCUSSION

We found a majority of *P. aeruginosa* isolates in a tertiary hospital in Nepal came from inpatients, men and patients aged >40 years. Although most patients recovered, there was, nevertheless, a high proportion of deaths. Although respiratory samples were the most common type, these were significantly less likely to host MDR or XDR isolates; most MDR and XDR

isolates came from sites open to active intervention by healthcare staff. Unlike several previous studies in Nepal,<sup>20,24,26,27</sup> none of the 19 antibiotics we tested were free of resistant strains of *P. aeruginosa*.

This study had arguably the largest sample size and tested more antibiotics than previous studies on *P. aeruginosa* in Nepal. It is thus a matter of concern that none of the AWaRe groups of antibiotics tested had any completely susceptible antibiotics. Trends from comparison of the results of the previous studies are hard to identify, with varying degrees of resistance over time and geography (Table 4). Nevertheless, we believe that resistance to antibiotics in Nepal is increasing, for at least two reasons: 1) we found no fully susceptible antibiotics across the three AWaRe classes, unlike several previous studies which looked at fewer antibiotics (including the latest study<sup>29</sup> with only six relevant isolates) and therefore had a greater chance of finding widespread resistance, and 2) we reported XDR isolates (22%; resistant to  $\geq 3$  and susceptible to  $\leq 2$  groups of antibiotics) for the first time.

All this indicates two aspects of AMR facing clinicians and public health specialists. First, inpatient prevalence, together with the resistant isolates found in samples from sites with active intervention, suggests that failure of infection control within the healthcare system is a substantial contributor to the spread and prevalence of *P. aeruginosa*. *P. aeruginosa* commonly causes nosocomial infection, which our data support, despite our limited clinical information.

Second, inpatients and invasive sites contributed most of the drug-resistant isolates, compounding the issue of infection control failure with possible inappropriate antimicrobial therapy. Such inappropriate antimicrobial therapy may contribute to the high death rate in our patients.<sup>30,31</sup>

The predominance of males and those over 40 years of age may reflect local disease patterns in general, patterns of exposure to *P. aeruginosa* or antibiotics or cultural approaches that favour men. Further work is needed to understand these aspects of AMR in Nepal. Furthermore, the notable prevalence of resistant strains in outpatients and emergency patients suggest poor infection control in other healthcare settings in the country, or community spread, or both. However, we did not find any report from Nepal on community aspects of *P. aeruginosa* infections.

Our levels of resistant strains among the Watch group of antibiotics are largely comparable to previous studies in Nepal (Table 4). This may reflect the widespread use of these easily available antibiotics without the prescriber knowing the infection status or the antibiotic susceptibility. The lowest resistant rates were found for two Reserve antibiotics, with a higher susceptibility rate among some Access antibiotics (amikacin and gentamicin), similar to some

other studies.<sup>28,32</sup> The fact that resistance is found to all antibiotics and across all three of the AWaRe groups indicates that widespread use still needs to be controlled and rationalised, both in hospitals and the community.

The increasing worldwide incidence of infections caused by MDR and XDR *P. aeruginosa* is a serious public health problem, as these infections are now a major threat to healthcare, even in well-resourced health systems such as the United States.<sup>33</sup> Resistant strains of *P. aeruginosa* are associated not only with high mortality, but also with increased resource utilisation,<sup>34</sup> putting further burdens on the limited resources available in low- and middle-income countries such as Nepal.

Our study, along with others, suggests that the development of amikacin and gentamicin resistance in *P. aeruginosa* strains is less common than for other antibiotics. The use of amikacin and gentamicin could thus provide a better chance of success in empirical therapy. However, that may only increase AMR<sup>35</sup> without other controls on the use and sale of antibiotics and improved hygiene practices.

The development of rapid, low-cost, point-of-care tests to identify the infectious organism and AMR pattern is imperative in order to rationalise the use of antibiotics in *P. aeruginosa* infections, as in the many other infections affected by increased AMR.<sup>36,37</sup> However, the main thrust in controlling AMR in Nepal remains the active and consistent implementation of the national AMR containment action plan.<sup>38-40</sup>

### **Strengths**

The present large study records the pattern and prevalence of AMR, MDR and XDR in every *P. aeruginosa* isolate over a 13-month period in a tertiary hospital in Nepal. By classifying the results of the study according to the WHO AWaRe groupings, and using this approach to compare the results of previous Nepali studies, the study provides an insight into the ongoing AMR situation in Nepal across the last decade. It also offers adequate information on the AMR pattern of these isolates to enable clinicians to choose the right empirical antibiotics in life-threatening conditions. Furthermore, it contributes to the rationale for 1) robust infection control procedures, 2) antimicrobial stewardship in both hospital and community healthcare settings, and 3) ongoing surveillance of infection and AMR patterns at both hospital and national levels.



### *Limitations*

We did not perform genotypic comparisons of the isolates, which would help identify clones and clusters that arise from nosocomial transmission. However, it could be argued that genomic diagnostics are not a substitute for phenotypic characterisation, and that depending entirely on genomics may result in erroneous diagnoses and result in therapeutic extrapolations.<sup>41</sup> Furthermore, this is a uni-centre study; although we compared our results with previous studies, a multi-centre study with a large sample size would have generated more reliable results. Finally, as ample informative data on clinical details of patients were not available, we could not assess any correlation between drug resistance and the severity of illness.

### **CONCLUSION**

Our study demonstrated a high prevalence of MDR and XDR *P. aeruginosa*, particularly among isolates from inpatients, which may lead to greater treatment costs, limited therapeutic options and adverse clinical outcomes, including high mortality. AMR is as much a public health threat in Nepal as elsewhere, with increasing resistance to last resort or Reserve antibiotics, as well as to the whole AWaRe spectrum, which must surely be a cause for concern. Early detection, consistent rational treatment approaches, continuous surveillance programmes, and aggressive infection control practices are needed by individual prescribers, hospital and community health centres, public health practitioners, veterinary partners and national governments to control the spread of these organisms.<sup>40</sup>

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Conflicts of interest: None declared.

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**Table 1** Characteristics of samples ( $n = 447$ ) and patients ( $n = 362$ ) with *Pseudomonas aeruginosa* attending Nepal Medciti Hospital, Lalitpur, Nepal, September 2018–September 2019

Characteristics	<i>Pseudomonas</i> -positive isolates	
	<i>n</i>	(%)
Sample type ( $n = 447$ )		
Respiratory*	203	(45.4)
Urinary†	120	(26.9)
Surgical/wound‡	75	(16.8)
Invasive§	49	(11.0)
Age group, years ( $n = 362$ )		
>40	229	(63.3)
≤63	133	(36.7)
Department ( $n = 362$ )		
Inpatients	181	(50.0)
Outpatients + daycare	128	(35.4)
Emergency	53	(14.6)
Hospital exit outcome (inpatient/emergency; $n = 236$ )		
Improved/discharged	200	(84.7)
Died	25	(10.6)
Discharged on request	9	(3.8)
Left against medical advice	2	(0.8)

\* Includes sputum, nasal/throat swab, bronchioalveolar lavage, suction tube.

† Includes urine, catheter tip, semen.

‡ Includes wound swab, pus, ear discharge.

§ Includes blood, bone marrow, biopsy, body fluid, central venous line tip.

**Table 2** Resistance pattern for AWaRe groups of antibiotics used for *Pseudomonas aeruginosa*-positive isolates attending Nepal Medciti Hospital, Lalitpur, Nepal, September 2018–September 2019, compared to previous reports from Nepal

AWaRe Group	Antibiotics tested	<i>N</i>	<i>n</i>	%
Access	Amikacin	375	97	(25.9)
	Gentamicin	351	103	(29.3)
Watch	Piperacillin/tazobactam	375	90	(24.0)
	Ceftriaxone/sulbactam	2	1	(50.0)
	Ciprofloxacin	402	148	(36.8)
	Imipenem	377	173	(34.9)
	Meropenem	375	141	(37.6)
	Ceftazidime	341	136	(39.9)
	Tobramycin	340	103	(30.3)
	Levofloxacin	200	92	(46.0)
	Ceftriaxone	173	100	(57.8)
	Cefotaxime	107	67	(62.6)
	Ofloxacin	101	55	(54.5)
	Norfloxacin	98	51	(52.0)
	Cefepime	84	42	(50.0)
	Cefuroxime	30	28	(93.3)
Reserve	Polymixin B	378	25	(6.6)
	Colistin	373	32	(8.6)
	Aztreonam	335	110	(32.8)

AWaRe = Access, Watch and Reserve.

**Table 3** Prevalence of multidrug-resistant and extensive-drug resistant isolates in samples and patients positive for *Pseudomonas aeruginosa* attending Nepal Mediciti Hospital, Lalitpur, Nepal, September 2018–September 2019

Characteristics	Total N	Multidrug-resistant isolates*					Extensively drug-resistant isolates†						
		N	%‡	cOR	P value	aOR	95%CI	n	%‡	cOR	P value	aOR	95%CI
Sample type§	447	190	42.5					99	22.1				
Urinary	120	59	49.2	Ref.		Ref.		37	30.8	Ref.		Ref.	
Respiratory	203	61	30.1	0.44	0.001	0.41	0.24–0.72	24	11.8	0.30	<0.001	0.29	0.15–0.58
Surgical	75	46	61.3	1.64	0.098	1.63	0.80–3.30	24	32.0	1.06	0.864	0.86	0.39–1.90
Invasive	49	24	49.0	0.99	0.982	0.83	0.37–1.86	14	28.6	0.90	0.771	0.78	0.32–1.91
Patients	362												
Age ≤40	133	57	42.9	Ref.		Ref.		26	19.6	Ref.		Ref.	
Age >40	229	78	34.1	0.69	0.096	1.00	0.61–1.65	40	17.5	0.87	0.621	1.21	0.67–2.21
Outpatients	128	33	25.8	Ref.		Ref.		15	11.7	Ref.		Ref.	
Inpatients	181	89	49.2	2.78	<0.001	3.22	1.90–5.44	43	23.8	2.35	0.009	2.63	1.35–5.12
Emergency	53	13	24.5	0.94	0.860	1.12	0.51–2.43	8	15.1	1.34	0.536	1.53	0.58–4.00

\* The multidrug-resistant phenotype is defined as *P. aeruginosa* resistant to  $\geq 3$  groups of antimicrobial agents.

† Extensively drug-resistant bacteria are those organisms resistant to  $\geq 3$  and susceptible to  $\leq 2$  groups of antibiotics.<sup>8,18</sup>

‡ Row percentage.

§ Urinary samples = urine, catheter tip, semen; respiratory samples = sputum, nasal/throat swab, bronchioalveolar lavage, suction tube; surgical samples = wound swab, pus, ear discharge; invasive samples = blood, bone marrow, biopsy, body fluid, central venous line tip.

aOR = adjusted odds ratio; cOR = crude odds ratio; CI = confidence interval; Ref. = reference.



**Table 4** Comparison of resistance patterns for AWARe groups of antibiotics used for *P. aeruginosa* positive isolates in reports from Nepal, sampled from 2010 to 2020

	Bhandari, 2012	Anil & Shah, 2013	Baniya, 2017	Thapa, 2017	Baral, 2019	Ansari, 2016	Yadav, 2020	Shrestha, 2019	This study	
<b>Sampling characteristics</b>										
Years of sampling	2010–2011	2012	2013–2014	2014	2014–2017	2015	2017	2018	2018–2019	
Duration of sampling, months	6	6	11	6	36	6	12	6	13	
Sample size	66	145	85	54	313	178	161	90 (29*)	Median 340 [IQR 104–375]	
Equivalent sample size for 12-month study	132	290	93	108	104	356	161	180 (58*)	Median 314 [IQR 96–346]	
<b>Antibiotics by AWARe Group (% resistant)</b>										
Access	Amikacin	74	17	51	25	17	16	37	17 (35*)	26
	Gentamicin	93		48	46	23	28	56	20 (45*)	29
Watch	Piperacillin/tazobactam	17		46	21	20	39	61	41 (75*)	24
	Ceftriaxone/sulbactam									50
	Ciprofloxacin	53	52	39	54	23	51	88	34 (72*)	37
	Imipenem		0	69	8		40	58	9 (6*)	35
	Meropenem	35				26	40	61		38
	Ceftazidime	94		49		58	73	87	83 (86*)	40
	Tobramycin						20			30
	Levofloxacin							77		46
	Ceftriaxone	94	69			65	92			58
	Cefotaxime					71	57			63
	Ofloxacin			37	54			86		55
	Norfloxacin									52
	Cefepime			22		48		84	58 (86*)	50
	Cefuroxime									93
	Cefixime	99				91				
Amoxicillin-clavulanic acid					87					
Chloramphenicol		0								
Reserve	Polymixin B	29		0	25	0	0	0	0 (0*)	7
	Colistin			84			0	0	0 (0*)	9

Aztreonam				33					33
Multidrug-resistant prevalence, %	89	21	66	44	60	—	73	32 (79*)	42
Number of antibiotics tested‡	9	8	9	9	11	14	12	9	19

\* Biofilm producers.

† No information.

‡ Includes non-AWaRe antibiotics not listed in the table.

AWaRe = Access, Watch and Reserve; IQR = interquartile range.