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Extended spectrum and AmpC beta lactamases - Producing *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* in Pediatric Care in Nepal

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Abstract

Extended-spectrum β-lactamases (ESBLs) and AmpC β-lactamases are key mechanisms behind β-lactam antibiotic resistance, particularly in Gram-negative bacteria. In Nepal, antibiotic-resistant bacterial infections pose a growing public health concern among children. However, there is limited pediatric-focused evidence on ESBL and AmpC β-lactamase production. To address this gap, a cross-sectional, hospital-based study assessed β-lactamase-mediated resistance among pediatric patients at the International Friendship Children's Hospital, Nepal, between August 2017 and February 2018. A total of 1,139 clinical samples (880 urine and 259 pus) were processed using standard microbiological techniques. Of these, 31 isolates of Klebsiella pneumoniae (urine) and 10 of Pseudomonas aeruginosa (pus) were identified. Phenotypic detection of ESBLs was performed using the combination disc test and AmpC β-lactamase production was assessed using the cefoxitin–cloxacillin double-disc synergy test and found 32.2% of and 40% of positive ESBL, while 46.2% and 33.3% positive AmpC β-lactamases for K. pneumoniae and P. aeruginosa isolates respectively. Co-production of ESBL and AmpC occurred in four K. pneumoniae and one P. aeruginosa isolate. Multidrug resistant (MDR) and Extensively drug resistant (XDR) were seen in 35.5% and 16.1% of K. pneumoniae and 40% and 20% of P. aeruginosa isolates, respectively. Meropenem, piperacillin-tazobactam, and gentamicin were the most effective antibiotics. These findings underscore the urgent need for routine resistance surveillance and evidence-based antibiotic policies to support effective pediatric infection management in Nepal.

Keywords: Multidrug-resistant (MDR), Extensively drug-resistant (XDR), Extended-spectrum β-lactamases (ESBL), AmpC β-lactamase, *K. pneumoniae*, *P. aeruginosa* , Children

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Introduction

Antibiotic resistance is a growing global health concern, especially in medical settings complicating the treatment of bacterial infections. Extended-spectrum β-lactamases (ESBLs) and AmpC β-lactamases are key mechanisms behind β-lactam antibiotic resistance, particularly in Gram-negative bacteria. They are particularly more resistant than Gram-positive bacteria due to their structural and genetic traits and diverse resistance mechanisms, including enzyme production (such as ESBLs, AmpC, MBL (Metallo-β-lactamase), efflux pumps, and reduced membrane permeability [1].

ESBLs first identified in 1983, are capable of breaking down oxyimino-cephalosporins and monobactams, but not cephamycins or carbapenems. They have been found in various Enterobacteriaceae and Pseudomonadaceae globally, and most commonly detected in *Klebsiella pneumoniae* and *Escherichia coli* [2]. Organisms that produce ESBLs are considered resistant to all penicillins (except temocillin), most cephalosporins (except cefoxitin and cefotetan), and aztreonam [2]. Although ESBLs are reported worldwide, their prevalence varies greatly between regions, however the actual rate is hard to determine due to inconsistent testing and reporting practices [3,4]. Likewise, AmpC β-lactamases are another important class of resistance enzymes which hydrolyze a broad spectrum of β-lactams, including cephamycins and β-lactam/β-lactamase inhibitor combinations, and are not inhibited by agents such as clavulanate or tazobactam [5]. These enzymes can be chromosomally encoded or plasmid-mediated, with the latter enabling rapid horizontal gene transfer among Enterobacteriaceae [6,7]. Such enzymes make bacteria often multidrug-resistant and associated with nosocomial infections [8]. *K. pneumoniae* is a major cause of hospital-acquired infections such as septicemia, urinary tract infections, and pneumonia in neonates and young children, while *Pseudomonas aeruginosa* is a leading cause of wound infections and is known for producing multiple virulence factors and resistance mechanisms, including inducible cephalosporinases and acquired β-lactamases like ESBL

and AmpC [12,13].

Children, particularly neonates and infants, are highly vulnerable to infections caused by resistant pathogens due to their immature immune systems, frequent hospitalizations, and higher exposure to antibiotics. [9] In low-resource settings such as Nepal and South Asia, this vulnerability is further amplified by limited infection control infrastructure, overcrowded hospitals, and variable antibiotic stewardship practices. Recent studies from the region report a high and increasing burden of ESBL- and AmpC-producing Gram-negative organisms in children, with pediatric studies from Kathmandu and Bhaktapur showing ESBL prevalence ranging from 24% to over 40% among urinary and bloodstream isolates, frequently exhibiting multidrug resistance [9,10,11].

Determining the prevalence of ESBL- and AmpC-producing organisms in children helps guide empiric therapy, as limiting third-generation cephalosporins can reduce selection pressure, and β-lactam/β-lactamase inhibitor combinations or cefepime remain effective options [5,14]. Despite global concern, there is limited local data on the prevalence and resistance profiles of ESBL and AmpC producers in pediatric populations in Nepal [15]. This study aims to address this gap by determining the occurrence of co-existence of both enzymes, ESBL and AmpC in pediatric isolates, providing updated, clinically relevant data to inform treatment and antimicrobial stewardship in children.

Materials and methods

Study Design, Setting, and Population

A hospital-based prospective cross-sectional study was conducted in the Microbiology Department of International Friendship Children's Hospital (IFCH), Maharajgunj, Kathmandu, from August 2017 to February 2018. A total of 1,139 clinical samples - 880 urine and 259 wound/pus swabs—were collected from children under 12 years of age. Only properly collected samples showing significant bacterial growth were included, while contaminated, duplicate, leaked, or antibiotic-pretreated samples (>48 hours) were excluded. Relevant clinical history was obtained from patients or guardians, and quality control was maintained using standard strains, including *E. coli* ATCC 25922.

Bacterial isolation and identification

Samples were processed using standard microbiological methods. Identification of *K. pneumoniae* and *P. aeruginosa* was performed using conventional biochemical tests and Bergey's Manual of Systematic Bacteriology (**Figure: 1, 2**).

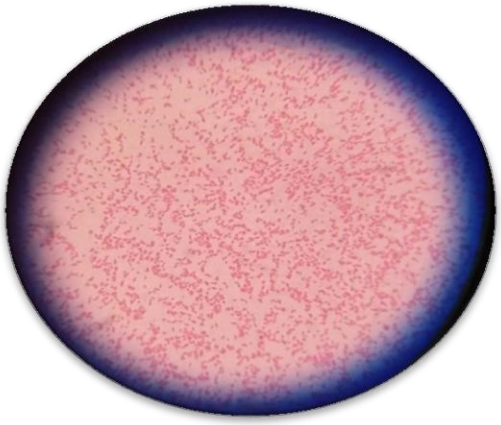

Figure 1. Photomicrograph of *Klebsiella pneumoniae.*

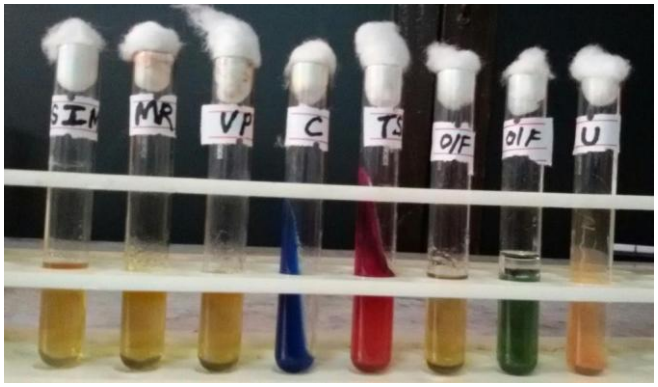

Figure 2: Biochemical test of *P. aeruginosa* (From left to right: SIM sulphite "– ve"; Indole "- ve"; Motile; MR "- ve"; VP "- ve"; Citrate "+ ve"; TSI Acid/no change; O/F "Oxidative"; Urease "- ve").

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was conducted using the Kirby-Bauer disk diffusion method on Muller Hinton Agar according to CLSI 2017 guidelines, with *E. coli* ATCC 25922 as a control. Antibiotics tested included cotrimoxazole, ciprofloxacin, levofloxacin, ceftazidime, gentamicin, meropenem, ceftriaxone, cefoxitin, nitrofurantoin, and piperacillin-tazobactam for *K. pneumoniae*, and ciprofloxacin, levofloxacin, ceftazidime, gentamicin, meropenem, cefoxitin, and piperacillin-tazobactam for *P. aeruginosa* . Multidrug-resistant (MDR) and extensively drug-resistant (XDR) isolates were classified according to international criteria [16].

Phenotypic confirmation of ESBL and AmpC production

ESBL production was screened using ceftazidime and confirmed with ceftazidime-clavulanate combination disks, where an increase in the inhibition zone ≥5 mm indicated positivity [4]. AmpC production was screened with cefoxitin and confirmed using the cefoxitin-cloxacillin double disk synergy test, with an increase in zone diameter ≥4 mm indicating AmpC positivity

(**Figure: 3, 4**) [17,18].

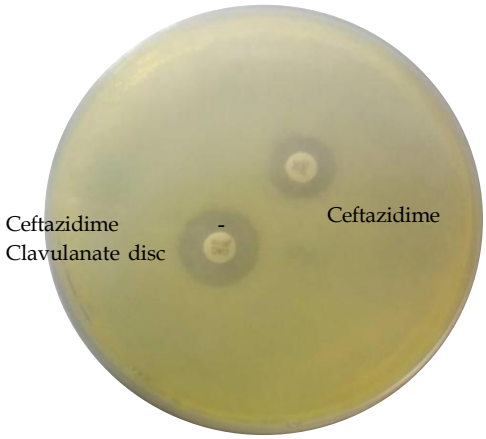

Figure 3. Confirmation test of ESBL producing *K. pneumoniae* showing increase in size of ceftazidime - clavulanate disc (≥5mm) than ceftazidime.

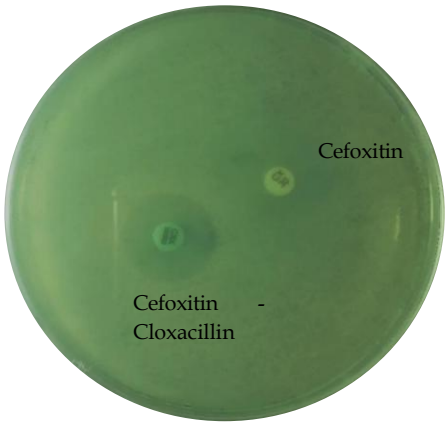

Figure 4. Confirmation test of AmpC producing *P. aeruginosa* showing increase in size of cefoxitin-cloxacillin (≥4mm) than cefoxitin**.**

Data Management and Statistical Analysis

Data were entered and analyzed using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp, Armonk, NY, USA). Descriptive statistics were used to summarize sample characteristics and resistance patterns, while inferential statistics were applied to assess associations, with p-values reported where appropriate.

Result

Distribution of clinical sample, Culture Positivity and sex distribution

Among total 1,139 clinical samples (880 urine and 259 pus), 196 (17.2%) were culture-positive, including 82 urine isolates and 114 wound/pus isolates. Of 880 total urine samples, 494 were the male patients and 386 were female. Out of 82 positive urine culture, 8.5% (42/494) were from male and 10.4% (40/386) were from female. Of 259 total pus samples, 135 were the male patients and 124 were the female. Out of 114 positive wound/pus and swab samples, 47.4% (64/135) were from male and 40.3% (50/124) were from female. (**Table 1**)

Table 1. Distribution of culture-positive isolates by sample type and sex

Sample	Total samples (n)	Male (n)	Female (n)	Culture-positive (n, %)	Positive in males (%)	Positive in females (%)
Urine	880	494	386	82 (9.3%)	42 (8.5%)	40 (10.4%)
Pus/ Wound swab	259	135	124	114 (44.0%)	64 (47.4%)	50 (40.3%)
Total	**1,139**	**629**	**510**	**196 (17.2%)**	–	–

Urine Isolates: Among 82 positive urine samples, the predominant pathogens were *E. coli* (41, 50%), *K. pneumoniae* (31, 37.8%), *P. mirabilis* (6, 7.3%), and *C. freundii* (4, 4.3%).

Of 494 urine samples from male patients, 17 (3.44%) were positive for *K. pneumoniae*, while 14 (3.63%) of 386 female samples.

Wound/Pus Isolates

Of 114 culture-positive wound/pus samples, the majority were *S. aureus* (97, 85.1%), followed by *P. aeruginosa* (10, 8.8%), *E. coli* (3, 2.6%), *Proteus spp.* (2, 1.8%), and *Enterobacter* spp. (2, 1.8%). Among 259 pus samples, *P. aeruginosa* was isolated in 10 samples, 6 from males (4.4%) and 4 from females (3.2%).

The associations between patient sex and infections were not statistically significant ($p > 0.05$) for both *K. pneumoniae* in urine and *P. aeruginosa* in wound samples.

Age distribution of *K. pneumoniae* Urine Isolates and *P. aeruginosa* Pus Isolates

Table 2. Age distribution of *K. pneumoniae* Urine Isolates

Age Group (Year)	Total Patients (n)	Overall Culture Positivity	*K. pneumoniae*	*K. pneumoniae* rate (% of total patients)
≤2	370	38 (10.3%)	19	5.13% (19/370)
3-4	199	18 (9.0%)	5	2.50% (5/199)
5-6	117	9 (7.7%)	2	1.71% (2/117)
7-8	70	7 (10.0%)	5	7.14% (5/70)
9-10	65	5 (7.7%)	0	0%
11-12	59	5 (8.5%)	0	0%
Total	880	82 (9.32%)	31	-

Patients were categorized into six age groups: ≤2 years (n=370), 3-4 years (n=199), 5-6 years (n=117), 7-8 years (n=70), 9-10 years (n=65), and 11-12 years (n=59). Overall culture positivity rates were 10.3% (38/370), 9.0%

(18/199), 7.7% (9/117), 10.0% (7/70), 7.7% (5/65), and 8.5% (5/59), respectively, with no plausible difference across age groups. Among these, *K. pneumoniae* positivity was observed as 19 cases in ≤2 years, 5 in 3-4 years, 2 in 5-6 years, 5 in 7-8 years, and none in 9-10 or 11-12 years. (**Table 2**)

Patients were categorized into six age groups: ≤2 years (n=124), 3-4 years (n=44), 5-6 years (n=36), 7-8 years (n=24), 9-10 years (n=17), and 11-12 years (n=14). Overall culture positivity rates were 46.8% (58/124), 45.5% (20/44), 33.3% (12/36), 58.3% (14/24), 23.5% (4/17), and 42.9% (6/14), respectively, with highest and lowest occurrence in 7-8 and 9-10 years. Among these, *P. aeruginosa* was isolated from 3 cases in ≤2 years, 2 in 3-4 years, 2 in 5-6 years, none in 7-8 years, 1 in 9-10 years, and 2 in 11-12 years. (**Table 3**)

Table 3. Age wise distribution of *P. aeruginosa* Pus Isolates

Age Group	Total Patients (n)	Overall Culture Positivity	*P. aeruginosa* Cases	*P. aeruginosa* Rate (% of total patients)
≤2	124	58 (46.8%)	3	2.42% (3/124)
3-4	44	20 (45.5%)	2	4.55% (2/44)
5-6	36	12 (33.3%)	2	5.56% (2/36)
7-8	24	14 (58.3%)	0	0%
9-10	17	4 (23.5%)	1	5.88% (1/17)
11-12	14	6 (42.9%)	2	14.29% 2/14)
Total	259	114 (44%)	10	-

Antibiotic susceptibility Pattern and Multidrug resistance

Table 4. Antibiotic susceptibility pattern of *Klebsiella pneumoniae*

Antibiotic	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
Cotrimoxazole	15 (48.3)	0 (0.0)	16 (51.6)
Ciprofloxacin	20 (64.5)	0 (0.0)	11 (35.5)
Ceftazidime	15 (48.4)	0 (0.0)	16 (51.6)
Levofloxacin	23 (74.2)	0 (0.0)	8 (25.8)
Gentamicin	28 (90.3)	0 (0.0)	3 (9.7)
Meropenem	28 (90.3)	0 (0.0)	3 (9.7)
Ceftriaxone	15 (48.4)	0 (0.0)	16 (51.6)
Nitrofurantoin	16 (51.6)	1 (3.2)	14 (45.2)
Piperacillin–tazobactam	26 (83.9)	0 (0.0)	5 (16.1)
Cefoxitin	16 (51.6)	2 (6.5)	13 (41.9)

Meropenem and Gentamicin were most effective against *K. pneumoniae* (90.3% susceptibility), followed by Piperacillin–tazobactam. Cotrimoxazole, Ceftazidime, and Ceftriaxone showed high resistance (51.6%). (**Table 4**)

In *P. aeruginosa*, Meropenem and Piperacillin–tazobactam were found to be drug of choice with 80% susceptibility while they were found to be highly resistant (90%) to Cefoxitin. Eleven and five isolates of *K. pneumoniae* were found to be MDR and XDR respectively. Similarly, four and two isolates of *P. aeruginosa* were found to be MDR and XDR respectively. (**Table 5**)

Table 5. Multidrug resistance exhibited by isolates

Isolate	No. of isolates	MDR n (%)	XDR n (%)	Total n (%)
K. pneumoniae	31	11 (35.5)	5 (16.1)	16 (51.6)
P. aeruginosa	10	4 (40)	2 (20)	6 (60)

ESBL Production and Its Age wise distribution

Out of 31 *K. pneumoniae* isolates, 16 were ESBL screen-positive, with 10 (32.2%) confirmed as ESBL producers -7 from male patients and 3 from females. Among these 10 ESBL- producing isolates, the highest proportion (66%) occurred in the ≤ 2 years age group, followed by the 7–8 years group. ESBL occurrence was equal (50%) in the 3-4 and 5-6 years age groups. The statistically significant association between sex and infection by ESBL producing isolate was observed ($p<0.05$), chi-square (4.266). (**Table 6**)

Similarly, 10 *P. aeruginosa* isolates, 8 were ESBL screen-positive, and 4 (40%) were confirmed as ESBL producers - 3 from males and 1 female. Each of the 4 ESBL-producing isolates was found in a different age group: ≤ 2 years, 3-4 years, 5-6 years, and 11-12 years. (**Table 7**)

Table 6. ESBL production among Klebsiella pneumoniae

Gender	Screening test Producer	Confirmatory test – Producer n (%)	Confirmatory test – Non-producer
Male	8	7 (87.5)	1
Female	8	3 (37.5)	5
Total	16	10	6

Table 7. ESBL production among *Pseudomonas aeruginosa*

Gender	Screening test – Producer	Confirmatory test – Producer n (%)	Confirmatory test – Non-producer
Male	4	3 (75.0)	1
Female	4	1 (25.0)	3
Total	8	4	4

K. pneumoniae: Of the 10 ESBL producers, 66% were in ≤2 years, followed by 7–8 years; 3–4 and 5–6 years had equal (50%) occurrence. Similarly, of 4 *P. aeruginosa* ESBL producers, one occurred in each age group: ≤2, 3–4, 5–6, and 11–12 years (**Table 8**).

Table 8. Age wise distribution of ESBL producer among *K. pneumoniae* and *P. aeruginosa*

	K. pneumoniae				*P. aeruginosa*			
Age group	ESBL producer n(%)		ESBL non-producer n(%)	Total	ESBL producer n(%)		ESBL non-producer n(%)	Total
≤ 2	6	(66.7)	3 (33)	9	1	(33%)	2 (66.7%)	3
3-4	1	(50)	1 (50)	2	1	(50%)	1 (50%)	2
5-6	1	(50)	1 (50)	2	1	(50%)	1 (50%)	2
7-8	2	(66.7)	1 (33)	3	0		0	0
9-10	0		0	0	0		0	
11-12	0		0	0	1	(100%)	0	
Total	10	(62.5%)	6 (37.5%)	16	4 (50%)		4 (50%)	8

Table 9. Production of AmpC β-lactamase in *Klebsiella pneumoniae*

Gender	Screening test –Producer	Confirmatory test – Producer n (%)	Confirmatory test – Non-producer
Male	8	4 (50.0)	4
Female	5	2 (40.0)	3
Total	13	6	7

Table 10. Production of AmpC β-lactamase in *Pseudomonas aeruginosa*

Gender	Screening test – Producer	Confirmatory test – Producer n (%)	Confirmatory test – Non-producer
Male	6	2 (33.3)	4
Female	3	1 (33.3)	2
Total	9	3	6

Table 11. Age wise distribution of AmpC β-lactamase producer

	K. pneumoniae			*P. aeruginosa*		
Age group	AmpC β- lactamase producer	AmpC β- lactamase non-producer	Total	AmpC β- lactamase producer	AmpC β- lactamase producer	Total
≤ 2	4 (30.8%)	5 (38.4%)	9	0	2 (22.2%)	2
3-4	0	0	0	1 (11.1%)	2 (22.2%)	2
5-6	0	0	0	0	0	0
7-8	2 (15.4%)	2 (15.4%)	4	0	0	0
9-10	0	0	0	0	0	0
11-12	0	0	0	2 (22.2%)	2 (22.2%)	4
Total	6 (46.2)	7 (53.8%)	13	3 (33.3%)	6 (66.7%)	8

Table 12. Co-existence of ESBL and AmpC β-lactamase production in *K. pneumoniae*

ESBL	AmpC producer	AmpC non-producer	Total
Producer	4 (12.9%)	6 (19.4%)	10
Non-producer	2 (6.5%)	19 (61.3%)	21
Total	6	25	31

Table 13. Co-existence of EBSL and AmpC β-lactamase production in *P. aeruginosa*

ESBL	AmpC producer	AmpC non-producer	Total
Producer	1 (10%)	3 (30%)	4
Non-producer	2 (20%)	4 (40%)	6
Total	3 (30%)	7 (70%)	10

AmpC β-lactamase Production and its Age wise distribution

Out of 31 *K. pneumoniae* isolates, 13 were screened as AmpC β-lactamase producers, with 6 confirmed - 4 from males and 2 from females. (**Table 9**) Similarly, among 10 *P. aeruginosa* isolates, 9 were screened, and 3 were confirmed as AmpC β-lactamase producers. (**Table 10**) Among *K. pneumoniae*, out of six AmpC β-lactamase producing isolates, the highest proportion (30.8%) was detected in children ≤2 years of age, followed by 15.4% in the 7–8 years age group. For *P. aeruginosa* , out of three AmpC β-lactamase producing isolates, AmpC production was observed in children aged 3–4 years and 11–12 years. (**Table 11**)

Co-existence of ESBL and AmpC β-lactamase

In *K. pneumonia* and *P. aeruginosa* , the co-existence of ESBL and AmpC β-lactamase production was found in

four samples (12.9 %) and in one sample (10%) respectively. (**Table 12**.) In *P. aeruginosa* , the co-existence of ESBL and AmpC β-lactamase production was found in only one sample (10%). (**Table 13**)

Discussion

Distribution of clinical samples

Out of a total of 1,139 samples collected, 880 were urine samples and 259 were pus specimens. Of urine samples, 82 (9.3%) samples showed significant growth. Five different genera were isolated with *E. coli* being the predominant organism (50%), followed by *K. pneumoniae* (37.8%). These findings align with previous studies in Nepal [19], although the positivity rate in our study was slightly higher than that reported by Jha [20], possibly due to variations in age distribution, sample size, or hospital catchment characteristics.

Among pus or wound samples, 44% (114/259) showed significant bacterial growth. *Staphylococcus aureus* accounted for the majority of isolates (85%), followed by Pseudomonas aeruginosa (8.77%). The predominance of *S. aureus* is expected due to its role as a common skin colonizer [21], whereas the presence of *P. aeruginosa* likely reflects its environmental persistence and its association with opportunistic infections [22].

Culture Positivity and Sex Distribution

Out of 82 urine culture positive samples, positivity among male was 8.5% and 10.4% among female. Among the 31 culture-positive *K. pneumoniae* samples, positivity rates were comparable between males (3.4%) and females (3.6%), with a higher proportion observed among females under two years of age. This pattern is consistent with established pediatric UTI risk factors such as prior antibiotic exposure, disruption of periurethral flora, and voiding dysfunction [20]. Emerging evidence also suggests a possible association between low serum vitamin D levels and increased UTI susceptibility in children, although vitamin D status was not assessed in this study [23]. Out of 114 culture-positive pus samples, 47.4% were from males and 40.3% from females. The increased positivity in males may be partly attributed to a higher proportion of male samples in the study population. Additionally, males are generally more engaged in outdoor and physically demanding activities, which may increase the risk of trauma and subsequent wound infections [22].

Age wise distribution of *K. pneumoniae* Urine Isolates and *P. aeruginosa* Pus Isolates

The predominance of *K. pneumoniae* isolates in children ≤2 years (19/31 cases, 61.3%) aligns with prior reports of this pathogen as a common uropathogen in young children. (24, 25) Females showed higher positivity rates in this group (6.4% vs 4.3%), consistent with established gender differences in pediatric UTIs. (24) The unexpected peak in 7-8 years (7.14%) may reflect smaller sample size (n=70) rather than true biological trend. No significant age association was found ($p>0.05$), suggesting *K. pneumoniae* UTI risk is evenly distributed across pediatric ages in this cohort.

P. aeruginosa isolates from pus samples showed no clear age predominance, with cases distributed across groups despite highest proportional positivity in 11-12 years (14.2%, 2/14). This contrasts with reports emphasizing *P. aeruginosa* in younger children with severe infections, possibly reflecting community-acquired wound infections in older children rather than neonatal sepsis. (26) Males showed slightly higher positivity in ≤2 years (2.8% vs 1.8% in females), differing from typical UTI gender patterns but consistent with wound infection epidemiology. The non-significant chi-square despite low $p=0.024$ suggests limited power from small *P. aeruginosa* case numbers (n=10); larger studies are needed to clarify age-specific risk in pediatric pus infections."

Antibiotic Resistance Patterns and Multidrug resistance

Antibiotic susceptibility testing showed high resistance of *K. pneumoniae* to third-generation cephalosporins (51.6%) but remained susceptibility to gentamicin and meropenem (90.3%). Nitrofurantoin showed moderate effectiveness (51.6%), consistent with its use primarily in uncomplicated UTIs due to limited tissue penetration and potential side effects [27]. This highlights the need to choose antibiotics based on both local resistance patterns and clinical context. *P. aeruginosa* , the pus isolates showed highest resistance to (70%) ceftazidime and 90% cefoxitin. However, it was susceptible to other beta-lactams i.e., (80%) piperacillin-tazobactam, and meropenem, 60% Gentamicin and Ciprofloxacin. The high prevalence of multidrug-resistant organisms 35.5% in *K. pneumoniae* and 40% in *P. aeruginosa* and XDR strains (16.1% and 20%, respectively). Existence of high drug resistance observed in this study points towards negligence on patients part, incomplete treatment schedules, antibiotics misuse, self-prescription, misprescription, lack of regional antibiogram data, and limited knowledge about multidrug-resistant isolates and antimicrobial resistance among clinicians [28,29].

Compared to a study conducted at an organ transplant center in Nepal identified that 10% of 308 isolates were XDR, of which 18% were *K. pneumonia* [30]. The slightly

higher prevalence in this study may reflect differences in patient population, pediatric focus, sample size, and single-center design.

ESBL production and its Age wise distribution of ESBL

Higher level of drug resistance seen among *K. pneumoniae* is mediated by the production of different kind of β-lactamases primarily ESBL, AmpC, KPC and Metallo β-lactamases [31]. In this study, 32.2% of urinary *K. pneumoniae* isolates were ESBL producers, which is comparable to previous studies of Nepal [30,31] although higher than Chaudhary [32], this discrepancy may reflect differences in detection methods, molecular analysis, or patient populations. The rise in antibiotic resistance is largely driven by gene dissemination via horizontal transfer, particularly through integrons, which contributes to multidrug resistance and ESBL production in many Gram-negative bacteria, including *Pseudomonas* [33]. In pus/wound isolates, 40% of *P. aeruginosa* were ESBL producers, consistent with findings by Davodian et al. [34]. This high prevalence likely reflects selective pressure from frequently prescribed β-lactam antibiotics, which promotes the development of ESBL enzymes [35]. Among *K. pneumonia*, the ESBL production in present study was higher (87.5%) among males than that of females (57.5%), and the prevalence was higher in ≤ 2 and 7-8 years age group. This study contradicts the study of Shakya et al [36] in which positivity among female (66.7%) was higher than that among male (33%). The differences in the ESBL rates may be attributable to the geographic difference, antimicrobials stewardship program and infection control practices.

P. aeruginosa , the ESBL production was higher among male (75%) than among female (25%). Further, the prevalence was higher in 11-12 years age group. Positive ESBL screening results may sometimes be due to AmpC β-lactamase rather than true ESBL activity, as inducible chromosomal AmpC enzymes can be activated by clavulanate and mask synergy with the indicator cephalosporin, potentially resulting in false-negative ESBL detection [37].

AmpC β-lactamase production and its Age wise distribution

Resistance to cephalosporins is often caused by overproduction of AmpC β-lactamases [2]. In this study, 41.9% of *K. pneumoniae* isolates were resistant to cefoxitin, and 19.3% were confirmed AmpC positive, though false positives can occur in KPC-producing strains when inhibitor-based methods are used [2]. Genes encoding both ESBL and plasmid-mediated AmpC β-lactamases are typically carried on large multidrug resistance plasmids, and plasmid-mediated resistance particularly carbapenemase genes has been increasing rapidly in *K. pneumoniae* [38]. In *P. aeruginosa* , 30% of isolates were AmpC producers, with 90% showing cefoxitin resistance in screening tests.

In this study, AmpC β-lactamase–producing *K. pneumoniae* isolates were slightly more common in males (4/6) than females (2/6) and were most prevalent in children ≤2 years, followed by those aged 7–8 years. Among *P. aeruginosa* isolates, AmpC producers were also slightly more frequent in males (2/3) than females (1/3), and were observed in the 3–4 years and 11–12 years age groups, indicating that AmpC production can occur across a broad pediatric age range [39]. The slightly higher prevalence among males compared to females may reflect differences in healthcare exposure or other behavioral factors. The higher occurrence in younger children could be attributed to frequent hospital visits, prior antibiotic exposure, or immature immune responses, which are known risk factors for colonization and infection with multidrug-resistant organisms [40].

Co-existence of ESBL and AmpC β-lactamase

Co-existence of ESBL and AmpC β-lactamase production was observed in 12.9% of *K. pneumoniae* and 10% of *P. aeruginosa* isolates. Similar co-existence has been reported in other Gram-negative bacilli, likely due to plasmid-mediated dissemination of AmpC enzymes, sometimes alongside ESBLs. Such strains may also yield false-negative ESBL results because inducible AmpC enzymes can mask synergy with clavulanate [2]. Carbapenems remain the only β-lactam antibiotics reliably active against co-AmpC and ESBL producers; however, increasing carbapenem resistance primarily due to MBL production poses a growing concern [38].

These findings highlight important resistance mechanisms in pediatric pathogens. The novelty of this study lies in its combined analysis of urinary and pus/wound isolates in a pediatric population, which is rarely reported in Nepal, providing a comprehensive overview of pathogen distribution and detailed phenotypic profiling of ESBL, AmpC, MDR, and XDR isolates. By offering one of the few age-stratified antimicrobial resistance datasets from a tertiary pediatric center, this study fills an important evidence gap and provides crucial baseline data to guide empirical therapy and antimicrobial stewardship. However, the study has limitations, including its single-center, hospital-based design, the absence of molecular confirmation of resistance genes, and lack of clinical data such as prior

antibiotic exposure, comorbidities, or nutritional status, which may influence infection and resistance patterns. Despite these limitations, the study provides valuable insights into pediatric infections in Nepal and emphasizes the urgent need for strengthened antimicrobial stewardship, rational antibiotic use, and routine surveillance to guide clinical practice.

Conclusion

This study highlights a high burden of antimicrobial resistance in pediatric infections in Nepal, with ESBL and AmpC production among urinary and wound isolates. By analyzing both urine and pus specimens and examining age-stratified resistance patterns, it provides pediatric-focused insights from a Nepalese clinical setting. The findings underscore the need for routine screening, pediatric-specific antibiograms, strengthened antimicrobial stewardship, and robust infection control measures. Despite its single-center design and lack of molecular confirmation, the study provides valuable insights to guide clinical practice in addressing multidrug- and extensively drug-resistant infections in children.

Author's contribution

PN designed the study, Methodology and wrote manuscript. RB assisted with laboratory procedures and PN and BS performed statistical analysis. ST supervised the laboratory work. BS revised the draft and provided overall supervision of the study.

Competing interests

This study does not involve any competing interests

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Informed consent

Informed consent was taken during the collection of the samples.

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