Original Article

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Biofilm Production and Antimicrobial Resistance among Uropathogens in Pediatric Cases: a Hospital Based Study

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ABSTRACT

Background: The study was designed to provide account of etiological agents of urinary tract infection in pediatric patients and the antimicrobial resistance pattern plus biofilm producing profile of the isolates.

Methods: The prospective study was conducted in Alka Hospital, Nepal with 353 clean catch urine samples from children. It was obtained during July 2014 to January 2015 which were first cultured by semi-quantitative method, followed by antimicrobial susceptibility testing and biofilm production assay on Congo red agar. Multidrug- resistance, extensively drug- resistance and pandrug- resistance among isolates were considered as per international consensus.

Results: Out of 353 samples, 64 (18.13%) showed positive growth in culture, confirming urinary tract infection. *E. coli,* 44 (68.8%) was the predominant organism followed by *Klebsiella* spp. 6 (14.1%). Most *E. coli* were sensitive to amikacin (93.2%) followed by nitrofurantoin (86.4%), and highly resistant to ampicillin (95.5%). Of 64 isolates, 23 (35.93%) were found to be multidrug- resistant strains. Biofilm was produced by 36 (56.25%) isolates.

Conclusions: This study showed higher biofilm production and resistance to in-use antibiotics rendering ineffective for empirical use. Regular surveillance of resistance patterns should be done to regulate multidrug- resistant bugs and to ensure effective management of urinary tract infection in children in a tertiary care setups.

Keywords: AMR; antimicrobial resistance; biofilm; urinary tract infection; UTI.

INTRODUCTION

Urinary tract infection (UTI), an infection of urethra, bladder, ureters and/or kidney, has significant morbidity and mortality.[1,2]Outpatient morbidity of UTI in Nepal was 311,944.[3] UTIs affect at least 3.6% of boys and 11% of girls.[4] UTI can lead to terminal renal disease in children.[5] Epidemiological reports point *Escherichia coli* as the most common cause of UTI in children.[6,7]

Biofilm, which account 80% of infections, is a sessile cells embedded in extracellular matrix.[8,9] The matrix assist in exhibiting an altered phenotype e.g. increased antimicrobial resistance (AMR).[10] Biofilm thus boosts pathogenicity of uropathogenic *E. coli* (UPEC).[11]

AMR, i.e. resistance against previously effective antibiotics, is the global threats to human health.[12,13] AMR increases morbidity, mortality, health-care cost and hospitalization.[14] Antibiotic choice should be based on AMR profiles of local strains varying temporally and geographically.[15]UTI in children is treated empirically to avoid complications.[16] Biofilm associated AMR has burthen the management of UTI. The study was done to insight uropathogens in pediatric UTI patients along with their AMR pattern and biofilm producing profile.

METHODS

The prospective study was conducted in the department of Microbiology of Alka Hospital, Lalitpur, Nepal; a tertiary level hospital. A total of 353 clean catch urine samples from children under 13 years of age were processed during the study period (July 2014 to January

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Urine culture was performed on routine culture media by semi-quantitative method as described in World Health Organization (WHO) manual.[17] Briefly, 1µL of urine was inoculated on MacConkey and blood agar plate by streaking using calibrated loop (2mm), and incubated aerobically for 18-48 hrs at 37°C. Growth of 100 colonies or more, i.e. 10^5 colony forming units (CFU)/mL urine, was considered as positive growth. Isolation and identification of microorganisms were done by standard microbiological operation procedure as described in the Manual of Clinical Microbiology.[18]

Antimicrobial susceptibility testing of all isolates against in-use antibiotics was performed by Kirby-Bauer disc diffusion method and interpretation of the results was done as described by Clinical and Laboratory Standard Institute (CLSI) 2013.[19] Antibiotic discs used were amikacin (30µg), ampicillin (10µg), cefepime (30µg), cefoxitin (30µg), cephalexin (30µg), ciprofloxacin (5µg), cotrimoxazole (1.25/23.75µg), gentamicin (10µg), imipenem (10µg), nitrofurantoin (300µg), norfloxacin (10µg), ofloxacin (5µg), piperacillin (100µg), and vancomycin (30µg). Organisms resistant to three or more classes of antibiotics were considered to be multidrug resistance (MDR). Similarly, extensively drug- resistance (XDR) and pandrug- resistance (PDR) organisms were considered as per international consensus.[20]

Detection of biofilm producers among isolates was done by culturing on Congo red agar. Intensity of color change of Congo red agar on microbial growth is proportional to the biofilm production by the organisms. Considering the intensity of color change, organisms were categorizedas strong biofilm producers, moderate biofilm producers, weak biofilm producers and biofilm non producers.

Ethical approval was obtained from Ethical Review Board, Nepal Health Research Council (NHRC), Nepal (Reg. no. 147/2014). Written informed consent was obtained from the guardian of children before enrolling to the study.

RESULTS

A total of 353 clean catch urine samples from children, under 13 years of age, were included under the study. The median age of children was 4 years (Interquartile range=5 years) with boys to girls ratio of 1:1.37. Samples from 64(18.13%) children showed positive growth in culture with boys to girls ratio of 1:1.21. UTI was more frequent in Indo-Aryan race compared to that in Sino-Tibetan race with ratio of 1.9:1 (Table 1).

Table 1. Distribution of patients.

	Total children	UTI confirmed children
Types of patients		
Out patients	282	55
In patients	71	9
Gender distribution		
Boys	150	29
Girls	203	35
Age distribution (Median age =4 years, Interquartile range =5 years)		
<3 months	33	13
3 months-3 years	129	27
>3 years	191	24
Race distribution		
Sino-Tibetan	155	22
Indo-Aryan	198	42
Total children	353	64

Ten different bacterial genera were isolated. *E. coli*, 44 (68.8%) was the predominant isolate followed by *Klebsiella* spp., 6 (14.1%) (Table 2).

Table 2. Pattern of bacterial isolates.

Gram's type of bacteria	Bacteria isolated	Frequency (%)
Gram-negative bacteria	*Acinetobacter* spp.	1 (1.6)
	C. freundii	1 (1.6)
	Enterobacter spp.	1 (1.6)
	E. coli	44 (68.8)
	Klebsiella spp.	6 (9.4)
	M. morganii	2 (3.1)
	Proteus spp.	3 (4.7)
	Providencia spp.	3 (4.7)
	P. aeruginosa	1 (1.6)
Gram-positive bacteria	*S. aureus*	2 (3.1)
	Total	64 (100)

In *E. coli* isolates, resistivity was highest to ampicillin (95.5%) and least to amikacin (6.8%). In other Gram-negative isolates, resistivity was highest to cefepime (33.3%) and least to amikacin (11.1%), gentamicin (11.1%), and piperacillin (11.1%) (Table 3).

Table 3. Antibiotic resistivity profile of isolates.

Class of antibiotics	Antibiotics	*E. coli* (n=44)*	Other Gram-negative isolates (n=18)*	*S. aureus* (n=2)*
Aminoglycosides	Amikacin	6.8%	11.1%	NT
	Gentamicin	36.4%	11.1%	0%
Beta lactam	Piperacillin	NT	11.1%	NT
Carbapenems	Imipenem	NT	0%	NT
Cephalosporins I	Cephalexin	44.2%	NT	NT
Cephalosporins II	Cefoxitin	NT	NT	100%
Cephalosporins IV	Cefepime	NT	33.3%	NT
Fluoroquinolones	Ciprofloxacin	40.9%	27.8%	0%
	Ofloxacin	38.6%	22.2%	NT
	Norfloxacin	NT	NT	0%
Glycopeptides	Vancomycin	NT	NT	0%
Nitrofurans	Nitrofurantoin	13.6%	NT	0%
Penicillins	Ampicillin	95.5%	NT	100%
Sulphonamide	Co-trimoxazole	61.4%	27.8%	0%

NT= Not tested, *Percentage calculated on n

Table 4. MDR profile and biofilm production among isolates.

Bacteria isolated	Biofilm production				MDR isolates	Total isolates
	Strong producer	Moderate producer	Weak producer	Non producer		
Acinetobacter spp.	0	0	1	0	0	1
C. freundii	0	0	0	1	0	1
Enterobacter spp.	0	1	0	0	0	1
E. coli	9	9	5	21	23	44
Klebsiella spp.	2	1	1	2	0	6
M. morganii	0	0	1	1	0	2
Proteus spp.	0	0	2	1	0	3
Providencia spp.	0	1	0	2	0	3
P. aeruginosa	0	1	0	0	0	1
S. aureus	0	0	2	0	0	2
Total	11	13	12	28	23	64

Of 64 isolates, 23 (35.93%) were MDR. All 23 MDR strains were *E. coli* isolates. Biofilm production in all isolates was higher i.e. 36 (56.25%) (Table 4).

Higher rate of antimicrobial resistance was found in biofilm producing *E. coli* strains. (Table 5)

Table 5. MDR profile of *E. coli* as per biofilm producing ability.

Antimicrobial resistance	Total isolates	Biofilm production			
		Strong producer	Moderate producer	Weak producer	Non producer
Non MDR	21	1	3	2	15
MDR	23	8	6	3	6
XDR	9	5	3	0	1
Possible XDR	11	2	3	1	5
PDR	0	0	0	0	0
Possible PDR	1	1	0	0	0

DISCUSSION

Increasing AMR among common isolates is posing difficulty in the management of UTI in pediatrics. Our study revealed high AMR and biofilm production among uropathogens. In our study, bacterial growths of $\geq 10^5$ CFU/mL in culture were considered as positive growth and thus UTI.[17] Only 64 (18.13%) samples showed positive growth. This signifies the need for review of in-use general microbiological methods and clinical examination.

Prior antibiotic treatment before submitting the urine sample, and other UTI like clinical conditions could be the factors responsible for low growth positivity.

In our study, the ratio of outpatients to inpatients was 3.97:1, while that in UTI confirmed children was 6.11:1. This implies higher prevalence of UTI in community children. In our study, boys to girls ratio was 1:1.21 in UTI confirmed children. UTI is more prevalent in girls, the natural epidemiology pattern.[21] Girls are more frequently affected by UTI due to colonizing colonic Gram-negative bacteria in urethra because of its anal proximity and shorter length.[22] Higher UTI prevalence in girls was also reported by other studies.[2,6] UTI was more frequent in Indo-Aryan race children compared to Sino-Tibetan race children with ratio of 1.9:1.

Gram-negative bacteria were predominant isolates 62 (96.88%) compared to 2 (3.12%) Gram-positive bacteria. Enterobacteriaceae family comprised of 54 (84.38%) bacteria. *E. coli* was the most predominant isolate 44 (68.75%), followed by *Klebsiella* spp. 6 (9.38%). This concords with reports from other studies.[6,7,15,21,23] UTI onsets when *E. coli* or other enterobacteria accesses and ascends the urethra. These organisms are among gastrointestinal tract flora colonizing periurethral area.[24]

The evolution of resistance against antibiotics is not a new phenomenon. And there is continual increase in reporting of AMR in recent years. In this study, *E. coli*, principal pathogen isolated, showed high resistivity to ampicillin (95.5%) and least to amikacin (6.8%). This concord with other studies.[6,7,15,21,23]Likewise, imipenem and amikacin were found most effective against other Gram-negative isolates and cefepime the least. Moreover, ciprofloxacin, co-trimoxazole, gentamicin and nitrofurantoin were found most effective for Gram-positive isolates. Development of MDR strains among common isolates of health care associated infection has been a major concern due to development of MDR strains among common isolates. Only 23 (35.93%) isolates were found MDR. This was similar to the other study.[2]

Co-trimoxazole, commonly used to treat pediatric UTI orally, was effective only against one third of isolates. Nitrofurans can still be relied upon as 1st line choice, the economical option too. But, higher resistance against fluoroquinolones renders it ineffective for 2nd line treatment. One of the most important findings in this study is the resistance to cephalexin with resistance rate of 44.2% for *E. coli* which portrays lower generation cephalosporins as unreliable empiric antibiotics. Empiric use of amikacin until the sensitivity pattern is available might be a reasonable choice. For avoiding complications, most children are treated empirically with antibiotics even before culture and susceptibility reports arrives.[21] Resistance against antibiotics is increasing nowadays in community patients. Also, lack of access to health facilities and inadequate public awareness are added worries.[25] In recent years, AMR is rising, self-medication being one but not the only reason.[26] Furthermore, antibiotic resistance is the holistic effect of inappropriate antibiotic policies, poor surveillance plus compliance, easy access to antibiotics, self-medication of antibiotics with poor adherence, suboptimal dosing, diagnostic errors, counterfeit drug of low quality, uncontrolled antibiotics use in agriculture and indiscriminate use of antibiotics without proper diagnosis or identification of causative agent.[25,27,28]

Biofilm production was higher in the isolates i.e. 36 (56.25%). In this study, biofilm production was found associated with high MDR, XDR and possible PDR. The biofilm stage determines the level of resistance. In initial phase, antibiotics and host immune system are the most effective.[10] Once the bacteria begin to secrete EPS and the attachment becomes irreversible, biofilm resist more against antibiotics and host immunity.[10] In addition, the spread of resistance markers and virulence factors can be promoted.[29] Combination therapy with macrolides (erythromycin, clarithromycin, and azithromycin) is recommended for biofilm-associated infections by different studies. Macrolides inhibits alginate thus averts matrix formation fort biofilm.[30]

Asia is regarded as one of the epicenters of AMR.[31] Inappropriate antibiotics treatment is the common healthcare practice, making this region the high risk area for AMR evolution. Our study highlights a high rate of MDR among uropathogens and a broad range of uropathogens in this setting. Variability of antibiotic resistance pattern as per different regions and recent continuous increase in resistant strains to new antibiotics has posed great difficulty in selecting appropriate antimicrobial agents for management of infection they caused. Also leading to increase in cost with need of new costly drugs as wells as prolonged hospital stay. Since, the study was limited to single tertiary setting, broader surveillance is must to disseminate local resistance profile of prevalent uropathogens. This will ensure the optimal empirical therapy of UTI. And macrolides should be supplement along with other antibiotic therapy to circumvent evolution of resistance by biofilm production. The small sample size from single tertiary setting outlines the limitation of the findings to generalize in the larger population. Also phenotypic characterization cannot be solely relied upon to describe the inherent cause of the AMR in population.

CONCLUSIONS

Majority of uropathogens causing UTI in pediatrics were Gram-negative bacteria with predominance of *E. coli* in a tertiary care setup. Amikacin and gentamicin were found effective against most of the Gram-negative bacterial isolates, including *E. coli*. One-third of the isolates were found to be MDR. The study demonstrates high resistance to commonly prescribed antibiotics. Thus, testing of antimicrobial susceptibility of uropathogens is preeminent before starting antibiotic therapy. Majority of the isolates were biofilm producers rendering higher antimicrobial resistance. Macrolides can be supplemented along with antibiotics.

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