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ORIGINAL

Study on antimicrobial resistance pattern of uro-pathogens at National Referral Hospital, Thimphu Bhutan

Estudio sobre el patrón de resistencia a los antimicrobianos de los uropatógenos en el Hospital Nacional de Referencia, Thimphu Bhutan

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Abstract

Introduction: Antimicrobial resistance among uro-pathogens is a global concern. A periodic analysis of the etiologic agents responsible for urinary tract infections and their antibiotic susceptibility patterns is fundamental for a fitted empirical treatment.
Methods: This is a cross-sectional study conducted at Clinical Microbiology unit of Jigme Dorji Wangchuk National Referral Hospital, Thimphu, Bhutan. Urine culture positive samples from January 2017 to December 2020 were included. The routine procedure for urine culture and the threshold cutoff for antimicrobial susceptibility were followed as per the Clinical Laboratory Standard Institute guidelines.
Results: There were a total of 10392 episodes of positive urine culture out of which 80.9 % were from females and 19.3 % were male patients. The mean age was 38.03±19.2 years old and the most affected age groups were 21-30 years old. *Escherichia coli* (79.57%) was the predominant uro-pathogens followed by *Klebsiella spp*. (9.64%). The highest antimicrobial resistance was observed with Amoxicillin (70.5%) and Cotrimoxazole (32.48%), and the least resistance was seen among Nitrofurantoin (10.3%) and Gentamycin (10.6%).
Conclusion: The most prevalent etiologic agents were *Escherichia coli* and *Klebsiella spp*. accounting about two-thirds of the community acquired urinary tract infections. This study suggests Nitrofurantoin and Gentamycin as the choice of agent for empiric treatment of urinary tract infections. In contrary, Amoxycillin should not be used as the first choice for treatment. A valuable data on the different uro-pathogens and its anti-microbial sensitivity patterns has been provided to ultimately benefit the physicians to guide empirical therapy for UTI.

Key words: Antimicrobial resistance, pathogen, urinary tract infection.

Resumen

Introducción: La resistencia a los antimicrobianos entre los uropatógenos es una preocupación mundial. Un análisis periódico de los agentes etiológicos responsables de las infecciones del tracto urinario y sus patrones de susceptibilidad antibiótica es fundamental para un tratamiento empírico adecuado.
Métodos: Este es un estudio transversal realizado en la unidad de microbiología clínica del Hospital Nacional de Referencia Jigme Dorji Wangchuk, Thimphu, Bután. Se incluyeron muestras positivas de urocultivo de enero de 2017 a diciembre de 2020. Se siguió el procedimiento de rutina para el urocultivo y el umbral de susceptibilidad a los antimicrobianos según las pautas del Clinical Laboratory Standard Institute.
Resultados: Hubo un total de 10392 episodios de urocultivo positivo, de los cuales el 80,9% correspondieron a mujeres y el 19,3 % a pacientes masculinos. La edad media fue de 38,03±19,2 años y los grupos de edad más afectados fueron de 21 a 30 años. *Escherichia coli* (79,57%) fue el uropatógeno predominante seguido de *Klebsiella spp*. (9,64%). La mayor resistencia antimicrobiana se observó con amoxicilina (70,5 %) y cotrimoxazol (32,48%), y la menor resistencia se observó entre nitrofurantoína (10,3 %) y gentamicina (10,6%).
Conclusión: Los agentes etiológicos más prevalentes fueron *Escherichia coli* y *Klebsiella spp*. representando alrededor de dos tercios de la comunidad infecciones del tracto urinario adquiridas. Este estudio sugiere que la nitrofurantoína y la gentamicina son los agentes de elección para el tratamiento empírico de las infecciones del tracto urinario. Por el contrario, la amoxicilina no debe utilizarse como tratamiento de primera elección. Se han proporcionado datos valiosos sobre los diferentes uropatógenos y sus patrones de sensibilidad antimicrobiana para beneficiar en última instancia a los médicos para guiar la terapia empírica para la ITU.

Palabras clave: Resistencia a los antimicrobianos, patógeno, infección del tracto urinario.

Introduction

Urinary tract infection (UTI) is one of the major public health problems globally, affecting approximately 150 million people worldwide. The hospitalization rate due to UTI is increasing annually and in United States alone, there were approximately 400000 cases with an estimated cost of 2.8 billion dollars[1]. This is further aggravated with the evolving antimicrobial resistance due to the overuse of empiric broad-spectrum antimicrobials for treatment of uncomplicated UTI[2]. For instance, many studies have reported the alarming antibiotic resistance rates of ampicillin, amoxicillin, nalidixic acid, ciprofloxacin, cotrimoxazole and norfloxacin among the *Escherichia coli* isolates[3,4]. In Bhutan, one of the most prescribed antibiotics for empirical therapy for UTIs is amoxycillin which was reported to be resistant up to 71.4%[5].

Urine culture is the standard method of choice for diagnosis of UTI and its laboratory surveillance data of etiologic agents and their susceptibility profiles is pertinent for empiric treatment[6]. However, in the resource limited settings, urine culture is not routinely performed, thus the data on most common uro-pathogens and its susceptibility patterns are often considered for empirical treatments. In addition, the guidelines state that the empirical therapy should be based on the available information on the etiologic agents and its antibiotic susceptibility testing data[7].

Currently, there is a scarcity of data in the country; hence this study aims to determine the prevalence of uro-pathogens and their susceptibility patterns at Jigme Dorji Wangchuck National Referral Hospital from 2017 to 2020. This information will aid in guiding the physicians for empirical treatment of uncomplicated UTI in the country.

Materials and method

Study Design

A hospital based retrospective cross-sectional study was conducted. The study was carried out in the microbiology unit of Jigme Dorji Wangchuck National Referral hospital, which caters to patients from the Western region of the country.

Study population and study duration

The study included all positive urine culture from 1st Jan 2017 to 31st December 2020. It involved only the review of the sample processing registers for that period. The routine procedure for sample collection and processing of urine samples were followed as per the existing protocols of the laboratory. Bacterial isolates were identified by routine procedures in the laboratory, antibiotic susceptibility testing and the threshold cutoffs were performed as per the CLSI guidelines[8,9]. The antibiotic disks concentration were; amoxicillin 10 mcg, cefotaxime 30 mcg, ceftriaxone 30 mcg, gentamicin 10 mcg, nitrofurantoin 300 mcg, cefazolin 30 mcg, ceftriaxone 30 mcg, nalidixic acid 30 mcg, norfloxacin 10 mcg, co-trimoxazole 25 mcg, gentamicin 10 mcg, nitrofurantoin 300 mcg, cefoxitin 30 mcg, vancomycin 30 mcg, penicillin G 10 units and erythromycin 15 mcg.

Data collection

Patient information; age, sex, laboratory results, including bacterial colony-forming unit counts, organisms cultured and antimicrobial susceptibility results were retrieved. No patient details, such as name were involved and confidentially were maintained.

Data analysis

Data were entered into Epi-Data version 3.1 (Epi-Data Association, Odense, Denmark) and was exported into Epi info version 7 (Centre for Disease Control and Prevention, US) for analysis. A p-value of <0.05 was considered significant.

Results and discussion

A total of 10392 uro-pathogens were isolated and identified over the four-year period 2017-2020. The most commonly isolated pathogens were *Escherichia coli* (79.5%; 95% CI=77.68-79.26%) followed by *Klebseilla spp*. (9.64%: 95% CI=9.17-10.30%) and *Staphylococcus saprophyticus* (3.17%, 95% CI=2.81-3.84%) (**Table I**). *Escherichia coli* were the pre-dominant uro-pathogen isolated over the four-year period. The prevalence of Gram positive uro-pathogens were 7.38% (n=767), and the most predominant species among these were *Staph. saprophyticus* (3.17%) and *Enterococcus spp*. (3.16%).

Among the total episodes of bacteremia involved in the study; 80.9% (n=8415) were female and 19.3% (n=2015) were male. The prevalence of bacteremia in female were statistically significant than the male group (*p* value = 0.0027). The mean age was 38.5 ± 19.8 years old. The most affected age groups were 21-30 years old followed by 31-40 years old. The figure I represents the age distribution of the participants.

The overall antimicrobial resistance for amoxycillin was 70.5 % followed by cotrimoxazole 32.48 % and the least were seen in nitrofurantoin 10.3% and gentamycin 10.6%. Among the two most common Gram negative uro-pathogens, the highest resistance was reported to amoxycillin 62.9 % followed by cortimaxazole 46.55 % (**Table II**). The least resistance was observed for gentamycin (12.69 %) followed by norfloxacin (22.81 %). *Escherichia coli* being the most pre-dominant organism had the highest resistance of 65.2 % to amoxycillin followed by cortimoxazole (40.9 %). The least resistance was seen in nitrofurantoin (6.8 %) followed by gentamycin (7.4%). The resistance rate of amoxycillin for *Escherichia*

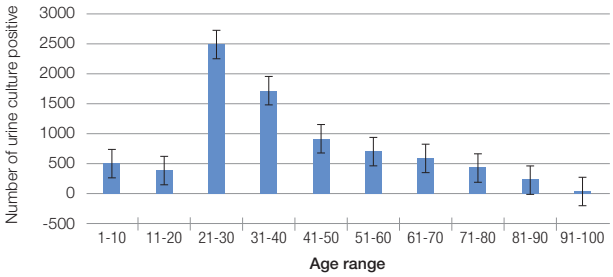
coli has been in a steep rise from 49.1% in 2017 to 76.3 % in 2020. The study also found that 34 % of the *Escherichia coli* isolated were resistant to the third generation cefalosporin antibiotics.

Among the top-two-Gram positive uro-pathogens, the highest resistance was observed in penicillin (61.08%) followed by tetracycline (26.7%). Least resistance was observed in nitrofurantoin (3.5%) followed by cotrimaxazole (21.7%) as presented in **table III**.

This study was conducted in an attempt to have an updated surveillance data of the etiological agents, its anti-microbial susceptibility patterns with respect to time and the local geographical area. This would provide valuable information for the physicians to guide empirical treatments in a resource limited country such as Bhutan.

We found that *Escherichia coli* was the most common uro-pathogen (79.5%) to cause uncomplicated UTI in patients visiting Jigme Dorji Wangchuck National Referral Hospital over the past four years (2017-2020).

Figure 1: Age distribution of patients presented with positive urine culture.



Studies at Iran, South Africa, India and Thailand have also reported *Escherichia coli* to be the most common uro-pathogen isolated which is in concordance with the current study[10-13]. These *Escherichia coli* strains are referred to as the uro-pathogenic Escherichia coli (UPEC) that possesses a variety of virulence factors including adhesions, toxins, iron acquisition factors, polysaccharide capsules and other invasions making it the most suitable candidate for invading the host cells causing UTIs[14].

Table I: Frequency of uro-pathogens isolated from 2017-2020.

Organism	2017		2018		2019		2020	
	N	%	n	%	N	%	n	%
Escherichia coli	2170	78.09	2567	78.91	1179	82.22	2313	79.05
Klebseilla spp	260	9.36	315	9.68	117	8.16	333	11.38
Enterococcus spp	73	2.63	151	4.64	41	2.86	74	2.53
Staph saprophyticus	134	4.82	89	2.74	43	3.00	62	2.12
Candida spp	16	0.58	29	0.89	11	0.77	33	1.13
Proteus spp	15	0.54	44	1.35	4	0.28	40	1.37
Pseudomonas spp	59	2.12	35	1.08	27	1.88	62	2.12
Acinetobacter spp	32	1.15	15	0.46	5	0.35	2	0.07
Enterobacter spp	17	0.61	5	0.15	5	0.35	4	0.14
Streptococcus spp	3	0.11	3	0.09	2	0.14	3	0.10
	2779		3253		1434		2926	

Table II: Percentage of resistance pattern of two most common Gram negative uro-pathogens.

Antibiotics	*Escherichia coli*					*Klebseilla spp*				
	2017	2018	2019	2020	Mean	2017	2018	2019	2020	Mean
Amoxycillin	49.1	67.2	68.2	76.3	65.2	71.0	40.5	98.3	93.7	75.9
Cefazolin	27.9	43.4	40.6	44.2	39.0	53.3	57.2	50.0	53.5	53.5
Cefalexin	26.4	43.7	40.5	-	36.9	33.7	23.2	44.8	13.2	28.7
Ceftraxione	24.7	37.8	34.9	38.7	34.0	46.7	55.3	49.1	46.8	49.5
Gentamycin	2.55	8.55	9.18	9.40	7.4	7.8	19.4	21.6	6.9	13.9
Nitrofurantion	4.97	10.9	6.82	4.46	6.8	29.8	13	27.6	6.0	19.1
Norfloxacin	19.8	38.9	38.7	31.8	32.3	19.2	13.8	35.3	10.2	19.6
Cotrimaxazole	35.7	47.4	42.90	37.4	40.9	48.2	51.8	42.2	40.2	45.6

Table III: Percentage of resistance patterns of two most common Gram positive uro-pathogens.

Antibiotics	*Staphylococcus spp*					*Enterococcus spp*				
	2017	2018	2019	2020	Mean	2017	2018	2019	2020	Mean
Ampicillin	-	-	-	-	-	28.7	30.5	43.9	50	38.3
Nitrofurantion	2.06	1.86	4.08	0	2.0	7.86	4.08	8.69	0	5.2
Norfloxacin	2.02	8.41	4.16	7.4	5.5	37.2	60.6	38.6	38.2	43.7
Penicillin	70.2	58.09	70	89.2	71.9	51.2	37.5	50	62.5	50.3
Tertacycline	6.34	9.27	3.7	-	6.4	-	47	-	-	47.0
Cotrimaxazole	9.45	14.01	10.6	3.48	9.4	28.5	57.1	16.6	-	34.1
Cloxacillin	75.3	42.7	27.5	18.5	41.0	-	-	-	-	-

Female are more susceptible than male due to the anatomic shortness of urethra and its proximity to the anus making it easy for the bacteria to ascend into the urinary tract[15]. Likewise, we reported a higher rate of bacteremia among the females as compared to the male subjects (80.9 vs 19.3 %). Similar studies in Thailand and India reported majority of the isolates from female patients[16].

The most affected age groups in our study were 21-30 years old. Similar results were observed by D Prakash (2021) and Zwane (2013)[11,12]. This is attributed to the sexual activeness during this age. The immense sexual intercourse could lead to abrasion in the vaginal tract leading to entry of pathogens causing UTI[17].

The laboratory-based surveillance data captures pertinent aspects on the effectiveness of the empirical treatment and is dependent on the prevalence of resistance among the most common causative pathogen[4]. Studies across other regions showed that *Escherichia coli* was the most frequent uro-pathogens isolated with resistance to amoxicillin; 88.3% in Saudi Arabia, 81.2 % in Iran, and cotrimoxazole by 50% and 79.2 %[16,18]. In the present study we found that amoxycillin resistance rate of 65.2% followed by cotrimoxazole 40.9% among the *Escherichia coli* isolates. Self-medication, availability over the counter and over prescribing of oral amoxycillin is a common practice in Bhutan which may have led to the increasing resistance[19].

The least resistance was seen among nitrofurantoin (10.3%) and gentamycin (10.6%), hence these antibiotics could be used for empirical treatment of UTI effectively. However, a periodic analysis and surveillance data is pertinent. This is because a report from elsewhere, found 78.71% nitrofurantoin resistance[12].

The resistance rate of amoxycillin among the *Escherichia coli* isolates was 49.1% in 2017 and 76.3% in 2020, with an increasing trend each year. This result implies that antibiotic resistance can change overtime and a proper antibiotic program can reverse an outbreak of resistant pathogen. For instance, prevalence of vancomycin-resistant Enterococcus (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA) and other Gram-negative Bacilli were all reduced when such plan was implemented at Methodist Hospital at Indiana[20]. Therefore, we suggest advocacy and sensitization works against irrational use of antibiotics as well as periodic community-based surveillance of antimicrobial resistance.

There are limitations in this study, it included only the Western part of the country and may not represent resistance patterns in other parts of the country. Furthermore, the knowledge on the antibiotic resistance among the uro-pathogens should be documented which will help in development of necessary interventions.

Conclusion

This study provides valuable and updated etiological agents and the antimicrobial susceptibility profiles of uro-pathogens that could be used to guide empirical UTI treatments in Thimphu. This study shows a large number of isolates resistance to amoxycillin and cotrimoxazole and hence it is advisable to provide advocacy and other enhanced sensitization works against irrational use of antibiotics. In addition, there is a need for periodic community-based surveillance of antimicrobial resistance patterns.

Ethical clearance

Permission for waiver of ethical clearance was sought from Research Ethics Board of Health, Ministry of Health, Thimphu, Bhutan via letter no. Reference No. REBH/Approval/2019/091.

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Competeing intrest

Declared none.

Authors contribution

AM and VC carried out the study design, proposal write-up, data analysis and drafting of manuscript. TD, RS and PW was involved with data collection and drafting of manuscript. TC did the data analysis and drafting of manuscript. All the authors read the final version of manuscript and accepted.

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