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The efficacy of antibiotics reduction activity against chronic kidney diseases caused bacteria

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Abstract

The aim of this study was to determine the efficacy of antibiotics activity against some chronic kidney diseases (CKD) caused bacteria. Three pediatric hospitals in Baghdad were included in this study which were Al-Mansour pediatric, Child's central and Al-Kadimiya. In this study some bacteria were isolated from children, then their susceptibility towards selected antibiotics were detected. Nitrofurantoin was the potent antibiotic whereas Gentamicin has the lowest effect against bacterial UTIs.

Key words: Chronic kidney diseases, Antibiotics, Reduction activity, Baghdad.

Introduction

Chronic kidney disease (CKD) is a worldwide public health problem progresses towards end stage renal disease (ESRD). In childhood, it is generally non-curable and progressive condition that leads to death by early adulthood (Nühl and Schaefer, 2008).

This problem is now recognized as a common condition (Pradeep *et.al*., 2008). Nephrotic syndrome (NS) is an important CKD in children which is characterized by the presence of proteinuria, hypoalbuminemia, hyperlipidemia and edema. (Bagga and Mantana, 2005). The other important CKD in childhood is chronic renal failure (CRF) which is a progressive irreversible destruction of the kidney tissues leading to the loss of renal function (Bagga and Mantana, 2005). CRF is the progressive, irreversible destruction of the kidney tissue by diseases that, if not treated by dialysis or transplant, will result in death of the patient (National Library of Medicine, National Institute of Health, 2004).

Urinary tract infection (UTI) is a common and important pediatric problem. The clinical importance of UTI is in the susceptibility to renal parenchymal damage developing to renal damage (Biassoni and Chippington, 2008). The knowledge of the causative agent of UTI in children is very important for effective treatment (Al-Harthi and Al-Fifi, 2008).

Certainly, the most commonly diagnosed UTIs are bacterial infections in infants and children (Song and Kim, 2008; Younis *et.al*., 2009). In children, UTIs represent an important problem because of both their frequency and the morbidity they sometimes generate (Florea, 2007).

The aim of this study was to determine the efficacy of antibiotics against CKD causing bacteria.

Materials and Methods

Antibiotic discs:

Both of sensitivity and resistance to antibiotics was recorded. The antibiotics used in this study were part of drugs commonly used in the treatment of UTI in children (Hull and Johnston, 2001; Beattie and Carachi, 2005). They were:

1. Ceftazidime (CAZ) 30 μg Bioanalyse.
2. Cefriaxone (CRO) 30 μg Bioanalyse.
3. Gentamicin (CN) 10 μg Bioanalyse.
4. Nalidixic acid (NA) 30 μg Bioanalyse.
5. Nitrofurantoin (F) 300 μg Bioanalyse.
6. Trimethoprime (Tr) 10 μg Bioanalyse.

Study groups

The pediatric patients were 26 of CRF and 36 of NS at the age of 1 to 12 years of both genders, who were outpatients and inpatients in the dialysis unit in Al-Mansour pediatric teaching hospital in The Medical City, Child's central teaching hospital and Al-Kadimiya pediatric hospital. The control group consisted of 26 children of both genders and at the same age range of the study groups.

Urine samples:

Urine collection is difficult in early childhood, and parents must be given adequate advice and supervision concerning how to collect a fresh, clean sample before antibiotics are started (International pediatrics, 2002). The Confirmed CKD pediatric patients by the nephrologists in the selected hospitals were considered as sick groups. The urine samples were collected in sterile containers or in urine bags in infants and small children (Polnay *et.al*., 2006). Specimens were taken from the patients and controls, and then transported to the library for general urine examination and culture.

Preparation of culture media and indicators

They were prepared according to the directions of manufacturing companies and were used for growing and identifying bacterial species.

Urine culture:

Urine culture is an investigation that allows identification of pathogen and its drug sensitiveness (Baka-Ostrowska, 2008). Urine samples were cultured on both blood agar medium and MacConkey agar medium as described by (Cavangolo, 1995) .Urine culture is the optimal method in UTIs diagnosis and detection of the causative agents. Any antibiotic therapy should not be taken before at least three days before urine culture (Robinson *et.al*., 1999).

In this study, the bacterial growth was considered significant as below (Sleigy and Timbury, 1983):

- Equal to or less than 30 colonies: non significant growth.
- Equal to 31-100 colony: moderate and significant growth.
- More than 100 colony: heavy growth.

The pure growth of more than 10^5 colony forming unit per ml of urine is usually defined, and the mixed growth usually indicates contamination. (Rudolph *et.al*., 2002). Then the bacterial isolates were identified.

Identification of bacteria that cause UTI

The cultural and biochemical characteristics defined the bacterial strains (Florea, 2007 ;Brooks *et.al*., 2007). The morphologic identification of the agent in stains of specimens light microscopy. (100x). The bacteria of urine cultures were identified by the morphological characteristics, biochemical tests and Gram stain, confirmed by Api 20E system. Then, the antibiotic sensitivity of isolates was tested by applying Kirby Bauer's disc diffusion method.

Antibiotics sensitivity test

The testing of the sensitivity to antibiotics is performed by the disc diffusion method (Kirby-Bauer's method) on Mueller-Hinton agar. The resulting inhibition zones have been measured by using a ruler then compared with standard inhibition zones determined by Clinical and Laboratory Standards Institute (2009). Both of sensitivity and resistance to antibiotics was recorded.

Statistical analysis

Data were translated into a computerized database structure. Statistical analysis was computer assisted using SPSS (2008). The charts were done by using curve estimation system (the quadratic mode).

The statistical significance of association between two variables within the same group was assessed by Chi-square. LSD was used in comparison between two different groups. p-value less than 0.05 was considered statistically significant.

Results and Discussion

Urinary tract infection occurrence with renal disease:

It had a significant association. The p-value was 0.042. The percentage of the positive cultures was 50% in the CRF patients, while it was 25% in the NS patients. Table (1). Yassin (1988) found that 20% of patients with renal problems were having UTI. Gulati *et.al*. (1996) and Ibadin and Abiodun (1998) recognized UTI in 40.26% 44.8% of NS pediatric patients respectively means that UTI is an important but often underdiagnosed infection in children with NS. Moorani, *et.al.* (2003) recorded that 12.5% of NS pediatric patients had UTIs because they were having either relapse or had recent onset of disease. In both NS and CRF patients, the positive cultures were 25% in Al-Mahdi (2004) study. Al- Bewyaney (2005) found that 25% of nephrotic children had UTIs; it is the same percentage in this study. Ali (2008) found a significant difference between diseases accompanied with UTI in children. In NS patients, the immunoglobulins are lost in proteinuria, especially in chronic glomerular disease (Falk *et al*., 2004) placing the child at risk of bacterial infection

and UTI. Moreover, Tokuda *et.al*. (2000) considered the CRF and ESRD patients as immunodificient. So, this may affect the immune system or make them susceptible to viral or bacterial infections (Abbass, 2007).

Table (1): Ocurrence of urinary tract infection within the type of CKD groups*

Type of CKD		UTI occurence		Total within CKD
		growth	No growth	
CRF	Count	13	13	26
	% within Type	50.0%	50.0%	100.0%
NS	Count	9	27	36
	% within Type	25.0%	75.0%	100.0%
Total	Count	22	40	62
	% within UTI	100.0%	100.0%	100.0%

*P- value = 0.042 (at 0.05 significance level).

The incidence significance of bacterial species with the type of CKD:

The positive cultures of urine samples formed 35.48% of the total count of patients' samples. Urine cultures of controls were negative. The bacteria were identified by morphological and biochemical tests according to Bergey's manual (Brenner *et.al*., 2005) confirmed by using api 20E system. The isolates bacteria as included in tables 1 and 2. The differences in the rates of infecting microorganisms probably due to multifactorial etiology of which different cultural habit, nutritional, socioeconomic and environmental factors and, also, might be due to age, gender or racial variations (Al-Ma'amoory, 2005). These results are in agreement with those of Telhe (2009) who isolated the same bacteria at the same classes from UTIs in patients with CRF. The primary reasons for *E. coli* and *Pseudomonas aeruginosa* predominance are their wide occurrence, ability to survive outside the human body for long periods and resistance to antibiotics (Wilson, 2008). Nosocomial UTI has the higher rate in many Iraqi hospitals. This is significantly associated with the duration of hospitalization, urinary catheterization or urinary endoscopy (Abbass, 2007).

Table (2): Distribution of bacterial isolates according to the type of renal disease*.

Bacterial species	CKD type		Total	Percentage
	CRF	NS		
E. coli	4	7	11	50 %
Pseudomonas aeruginosa	6	0	6	27.28 %
Klebsiella pneumoniae	2	0	2	9.09 %
Proteus mirabilis	1	1	2	9.09 %
Morganilla morgani	0	1	1	4.54 %
Total of positive cultures	13	9	22	35.5 %
No growth cultures	13	27	40	64.5 %
Total	26	36	62	100 %

*P- value = 0.031 (at 0.05 significance level).

The significant effect of selected antibiotics against bacterial species:

Based on the microbial sensitivity test results, drugs that are usually administered against uropathogens include Amoxicillin, Ampicillin and Cephalosporins, (inhibits cell wall synthesis), Aminoglycosides (inhibits on protein synthesis), Nalidixic acid (acts on DNA gyrase), and Nitrofurantoin (interferes with carbohydrate metabolism). However, many reports have indicated the presence of multi-drug resistance in organisms causing UTI (Mashouf *et.al*., 2009; Leonard *et.al*.,2009). *Enterobacteriaceae* members, particularly *E. coli*, are resistant to Amoxicillin and Ampicillin (Burg *et.al*., 2006). More seriously, ill children are initially treated with third-generation Cephalosporin or Aminoglycosides. Patients with renal impairment are particularly at risk for drug-related nephrotoxicity when using Aminoglycosides for prolonged periods of time (Leonard *et al*., 2009). Ciprofloxacin is not approved for use in children less than 18 years as it cause joints damage (Brooks *et.al*., 2007). In babies or ill children, an intravenous Cephalosporin would be given (Chaudhry and Harvey, 2001).

As shown in table (3) it is obvious that the antibiotic discs varied in their effects on the five types of bacteria. The significance was also different between cases according to the bacterial isolates and the drug used. Multiple drug resist-

ance is common and might be under the control of transmissible plasmids (Brooks *et.al*., 2007). Uropathogenic bacteria can resist the antibiotics by one or more of resistance mechanisms (Kayser *et.al*., 2005; Brooks *et.al*., 2007). Nitrofurantoin which had 0% of resistance, was the unique effective antibiotic on *E. coli*. Nitrofurantoin was the most active agent against this bacterium in the results of Al-Khazrachi (2001), Al-Mahdi (2004) and Al-Hemidawi (2005) in Iraq. Nitrofurantoin is only used in urinary tract infections, and it is effective against Gram-positive and Gram-negative bacteria (Kayser *et.al*., 2005). *E. coli* was 100% resistant to both of Ceftazidime and Cefteriaxone. This resistance could be due to lack of PBPs, the target cells, or poor permeation of bacteria by the drug (Brooks *et.al*., 2007). *Pseudomonas aeruginosa* isolates were the most potent ones against tested antibiotics in many references (Al-Khazrachi, 2001; Abid *et.al*. 2002; Al-Shaibani, 2004; Mashouf *et.al*., 2009).

Table (3): The significant effect of antibiotics and resistance rates of bacteria.

Antibiotics discs*	Coliform bacteria					
	E. coli	*Proteus mirabilis* *Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Morganella morganii*	p-value	
CAZ	100%	0%	100%	100%	100%	0.001
CRO	100%	100%	100%	100%	0%	0.001
CN	100%	100%	50%	100%	100%	0.063
NA	100%	50%	0%	100%	100%	0.003
F	0%	100%	0%	100%	0%	0.001
Tr	100%	0%	50%	100%	100%	0.003

*CAZ: Ceftazidime, CRO: Cefteriaxone, CN: Gentamicin, NA: Nalidixic acid, F: Nitrofurantoin, Tr: Trimethoprime.

A high resistance rates appeared towards Cefteriaxone and Ceftazidime (100% of each). The significant increase towards them was present in other studies (Al-Harthi and Al-Fifi, 2008; Tawfiq, 2005). Cephalosporins are widely used. Therefore, they encountered significantly raising resistance by *Klebsiella* (Bonnet, 2004). Strains of *Proteus mirabilis* vary greatly in antibiotic sensitivity. So, susceptibility should be guided by laboratory findings (Greenwood *et.al*., 2005; Brooks *et.al*. 2007).

E. coli was resistant to Gentamicin at a percentage of 100%. This disagreed with the findings of Abid *et.al*. (2002) and Al-Hemidawi (2005) in which it was low. Korshed (2005) recorded high rates of resistance towards Gentamicin (75%). Antibiotic resistance by *Pseudomonas aeruginosa* has probably developed by the transfer of R plasmids from other drug-resistant enteric Gram-negative bacteria (Ross, 1986). *Klebsiella pneumoniae* had a percentage of 50% resistance against Gentamicin. This sounds what was recorded by Abid *et.al*. (2002) in which the resistance was moderate. That may be attributed to the production of modification (Guven, 2004). The resistance percentage of *Proteus mirabilis* isolates was 100% towards Gentamicin. Al-Mahdi (2004), Ali (2008) and Al-Gherawi (2009) recorded moderate resistance of . *Proteus mirabilis* towards Gentamicin.

E. coli was resistant to Nalidixic acid at a percentage of 100%. The poor susceptibility o*f Pseudomonas aeruginosa* may result from the tendency to inactivate the antibiotics or prevent their intracellular accumulation to inhibitory level (Hancock and Speert, 2000). Nalidixic acid is very effective in *Klebsiella pneumoniae* UTIs (Brooks *et.al*., 2007). The Nalidixic acid resistance rate by *Proteus mirabilis* rate was 50%. They are inherently sensitive to Aminoglycosides, but resistance, which may be due to enzyme or non-enzyme mechanisms, is recently common (Greenwood *et al*., 2005). Nalidixic acid is useful as urinary antiseptic with low resistance rates (Brooks *et.al*., 2007). *E. coli* was resistant to Trimethoprime at a percentage of 100%. *E. coli* had recorded a high resistance to Trimethoprime (94.9%) in Al-Gherawi's study (2009).*Pseudomonas aeruginosa* multi-drug resistance is related to its propensity to develop resistance during therapy (Goering *et al*., 2008).

The Trimethoprime resistance by *Klebsiella pneumoniae* might be as a result of plasmid R transfer from other drug-resistant enteric Gram-negative bacteria (Fluit *et.al.,* 2001). *Proteus mirabilis* resistance was 0% against Trimethoprime. The study result may indicate a higher affinity of isolates for sulfonamides than for ρ-amenobenzoic acid (PABA) (Brooks *et.al*., 2007).

Morganella morganii was isolated from one case only, so the resistance pattern could be related to this strain. The variation of antibiotic susceptibility in *Morganella morganii* may attribute to genetic variation between isolates (Brooks *et.al*., 2007).

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