ISSN:2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 5, Issue, 07, pp. 5091-5095, July, 2015

International Journal of
DEVELOPMENT RESEARCH

Full Length Research Article

CHANGE IN ANTIBIOTIC SUSCEPTIBILITY PATTERN OF COMMON ORAL ANTIBIOTICS IN ESCHERICHIA COLI IN URINARY ISOLATES: AN ELEVEN-YEAR RETROSPECTIVE STUDY

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ARTICLE INFO

Article History:
Received 20th April, 2015
Received in revised form
15th May, 2015
Accepted 29th June, 2015
Published online 30th July, 2015

Key words:
E. coli,
UTI,
CLSI,
CLED,
TSI,
ATCC.

ABSTRACT

Introduction: Urinary Tract Infection (UTI) is the leading cause of bacterial infections. Billions of dollars are spent on a yearly basis to treat it empirically and fruitful results cannot be obtained unless we direct our efforts to a judicious choice of antibiotics and means to avoid the increasing bacterial resistance.[1, 2, 3, 7] We observed the duration after which an organism showed significant change in antibiotic susceptibility (considering 20% as a significant change). This analysis aids us in knowing when we can expect an organism to acquire resistance to a particular antibiotic and hence increasing the effectiveness in the management of UTIs, with reduced risk of resistance.

Methodology: 10891 urinary isolates of *Escherichia coli* were collected from1st January 2002 to 31st December 2012. Samples were collected by convenient sampling and both inpatient and outpatient samples were included.

Results: The resistance pattern of four common oral antibiotics was collected in this study. Average Percentage resistance to Ampicillin, Amoxicillin-clav, Ciprofloxacin, and Trimethoprim-Sulfamethazole were found to be 84%, 41%, 58% and 66.7% respectively. Ampicillin and Amoxicillin-calv did not show much change in resistance over the 11 year period.

Conclusion: Significantly high resistance is noted to all commonly use oral antibiotics for urinary tract infection, and strict measures need to be taken to avoid this. Ampicillin and Amoxicillin-calv did not show significant change in resistance pattern throughout the 11 year period. Hence, they can be used for relatively longer period of time as an empirical therapy in low resistance areas without the need to check yearly for change in susceptibility pattern.

INTRODUCTION

Infectious diseases put a major burden over a community. In all bacterial infections occurring throughout the world, Urinary Tract Infections are considered to be the most common. UTI is treated via empirical antibiotics selected according to local susceptibility pattern and putting major financial burden on society (Foxman, 2002; Florian *et al*., 2006 and Mohammad-Jafari *et al*., 2006). According to an estimate around 150 million UTIs occur yearly world-wide. Accounts for $6 billion health care expenditure yearly (National Medical Student Curriculum, 2012).

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According to the 1997 National Ambulatory Medical Care Survey and National Hospital Ambulatory Medical Care Survey, 7 million outpatient visits and 1 million ED visits and 100,000 hospitalizations are attributed to urinary tract infections (Foxman, 2002; Florian *et al*., 2006 and Patel, et al., 2012). Approximately 15% of all antibiotics prescribed in US are for treating Urinary Tract Infections, with estimate cost going over $1 billion (Florian *et al*., 2006). It is difficult to calculate actual incidence rate of UTIs because it is not a reportable disease. The rising incidence and cost are not the only problems we are facing now. Due to irrational use of antibiotics there has been a major increase in resistance to all of the first line antibiotics that were being used to treat UTIs (Björkman *et al*., 2006). This may further be attributed to self-medication practices (estimated around 6.3%) or medications being advised by a chemist, or due partially treated UTIs,

(Farooqi *et al*., 2000 and Sturm *et al*., 1997). Poor knowledge of Antibiotic provider, lack of standard guidelines for empiric treatment in a community, improper antibiotic prescription practices, insufficient training and supervision of health personnel, lack of access to rapid diagnostic facilities, inappropriate marketing of pharmaceuticals, absence of legislation regulating the quality, poor enforcement efforts to stop unauthorized dispensing of antibiotics are some of the common factors that lead to the development of Resistance to commonly used antibiotics (Emily Leung *et al*., 2011).In order to give proper empirical antibiotic prevalence, the resistance pattern of urinary pathogens should be known in the community (Linhares *et al*., 2000). Patients having community acquired UTI and patients those who have hospital acquired should be treated with empiric antibiotic as separate groups according to local antibiograms because these groups may show difference in susceptibility patterns. Hospital acquired UTIs show higher rates of resistance (Fleming *et al*., 2014).

In Karachi, Pakistan, *E.coli* and *Klebsiella* are the two most common pathogens causing urinary tract infections (Farooqi *et al*., 2000; Abdullah *et al*., 2012). Ciprofloxacin is very commonly prescribed antibiotic for empirical treatment of urinary tract infections in Karachi, Pakistan. Unfortunately, non-judicious use of antibiotics, has led to a high level of resistance against ciprofloxacin reaching up to 57.2 % in Gram negative and 48.7% resistance in Gram-positive bacteria found in urinary cultures (Abdullah *et al*., 2012). Antibiotic Resistance is not just an issue in Pakistan, rather, it is an issue worldwide. Resistance is not much different between genders (McGregor *et al*., 2013) and study conducted in South India showed increase in resistance with age; mostly because of improper use of antimicrobials (Mandal *et al*., 2012). Antibiotic Resistance is not a new problem. Major steps should be taken to deal with this dangerous and serious issue. The Six point policy package to combat the spread of antimicrobial resistance presented by WHO, should be followed. The rising antimicrobial resistance demands attention. One of the steps that need to be taken are that proper empirical antibiotic be given for the infection present at that particular point in time. It would not be appropriate to use same empiric therapy that was used based on previous susceptibility patterns. We should be aware when the uropathogen is suspected to show a change in susceptibility and change the empiric therapy accordingly. This duration may vary from one community to the other, based on the pattern of their antimicrobial use, or may vary with the type of infections. This will help us in selecting the most efficacious and cost friendly empiric therapy

MATERIALS AND METHODS

Sample Collection

This retrospective cross study was conducted from 1st January 2002 to 31st December 2012, in the Department of Clinical Microbiology of Dr. Ziaudin Medical University Hospital Karachi. 10891 urinary isolates of *Escherichia coli* were collected during this study from in-patient and out-patient. Samples were taken from both genders, above 20 years of age. All urine Clinical samples were collected by convenience sampling. All the duplicate clinical isolates were excluded from this retrospective study. Approval from the hospital ethical committee was obtained. Information was obtained from Hospital inpatient and outpatient records.

Sample Processing

All clinical samples of urine were received in sterile container without any urine preservative. Clean-catch Midstream urine samples were inoculated oncysteine lactose electrolyte deficient agar (CLED) by colony-count method, with the help of 0.001ml NiChrome wire loop, where Catheter samples, suprapubic samples and percutaneous nephrolithotomy were isolated on CLED agar by streaking method with 0.1 ml NiChrome wire loop. Plates were incubated at 37ºC in CO_2 incubator in ambient air for 24-48 hours, by applying standard microbiological techniques. The interpretation of result and workup on base of $\geq 10^5$ cfu's/ml urine in Midstream urine (Stamm *et al*., 1982), while in case of in-patient with pyelonephritis and leukocyte is present >10/HPF the $\geq 10^3$ should be count as significant growth (Wilson *et al*., 2004). Every single colony of Catheter samples, suprapubic samples and percutaneous nephrolithotomy samples will be count as significant growth. *Escherichia coli* were identified using conventional techniques (colony morphology, gram staining, positive Indole test and Fermentation of sugars on Triple sugar iron (TSI) Medium) and API-20E® (Koneman *et al*., 2006).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed on Muellar Hinton agar (MHA) medium (oxide Ltd., England) using modified Kirby Bauer's disk diffusion method according to clinical and laboratory standards institute (CLSI) guidelines (NCCS M100-S8. 1998). A 0.5 McFarland equivalent suspension of organism was inoculated onto an MHA plate, The antimicrobial discs were used, Amikacin 30 ug, Amoxycillin/Clavolanic Acid 2:1 30ug, Ampicillin 10 ug, Cefixime 5 ug, Trimethoprim 25ug, Cefotaxime 30ug, Nitrofurantine 300ug, Gentamicin 10 ug,, Ofloxacin 5 ug, Imipenem 10 ug, Meropenem 30 ug, Cefipime 30 ug, Tazobactam 110 ug, Cefpirome 30 ug, Carbinicilin 100 ug, Ceftazidime 30 ug and nitrofurantoin 300 ug. Local antibiotics Polymyxin B 300 ug, Tigecycline 15ug, Colistin sulphate 10 ug against gram negative bacilli.

American Type of Culture collection (ATCC) Controls will be used to check the quality of media and antibiotic disc. *E.coli* ATCC 25922 (Beta-lactamase negative) strains were used as a control organism. All the Gram-negative bacteria isolated from these clinical samples were tested for ESBL production by using four disks (concentration in µg) ceftazidime (30), ceftazidime/clavulanic acid (30/10), cefotaxime (30), and cefotaxime/clavulanic acid (30/10) and interpreted as per NCCLS guidelines (National Committee for Clinical Laboratory Standards, 1998).

Data Analysis

Data was analyzed by using Statistical Package for Social Sciences (SPSS) software version-17.0. The Resistant of All antibiotic in *Escherichia coli* were calculated and expressed in percentages.

RESULTS

The data of a total of 10891 urinary isolates of *E. coli* was obtained, over an eleven-year period, from both in-patients and out-patients, at a tertiary care hospital in Karachi, Pakistan. The resistance pattern of four common oral antibiotics was collected during this study. % resistance to Ampicillin varied from 76% to 88% with average of 84%, Amoxicillin and clavulanic acid resistant varied from 31% to 70% with average of 41%, Ciprofloxacin varied from 37% to 76% with average of 58% and trimethoprim sulfamethazole varied from43% to 88% with average of 66.7% (as shown in Figure 1).

DISCUSSION

Urinary Tract Infections are common in tertiary care hospitals especially in developing countries where there is breech in infection control practices. Various factors play role in acquiring this nosocomial infection such as improper care of urinary catheter, lack of use of personal protective equipments such as gloves and lack of hand washing practices (Kalsi *et al*., 2003; Saint *et al*., 2008). Common organism causing UTI are mostly intestinal bacteria flora and *E.coli* is most prevalent (Iqbal *et al*., 2002; Ali *et al*., 2001). UTI commonly occur in women than men and this is due to female urinary tract anatomy, it is estimated that half of women have at least one infection at some point in their life (Harrington *et al*., 2000; Hooton, 2000 and Urology Clinics of North America, 2009). Complications associated with UTI include pyelonephritis, cystitis and sepsis which have high impact on morbidity and mortality of patients (Cunningham *et al*., 1994).

Prevalence of urinary tract infection in Pakistan is 28% in those suspected with UTI (Jai Pal Paryani *et al*., 2012). The drugs commonly used to treat UTI include Beta lactam group of drugs (such as ampicillin, amoxi-clav, and ceftriaxone), Fluroquinolones (which include Ciprofloxacin) and Folate reductase inhibitors (which include Trimethoprim and Sulfamethaoxazole) (Gupta *et al*., 2010). Non-judicious use of antibiotics, over counter sale, lack of antibiotic steward shipping and inappropriate dosing and duration of therapy are all important factors which are causing development of resistance against commonly used antibiotics (Khalil Ahmed and Imran., 2008; Sturm *et al*., 1997). In our study we have calculated % resistance to Ampicillin throughout 11 years which remained almost unchanged and varied form 76% to 88% with average of 84% (Figure 2).

The Amoxicillin and Clavulanic acid resistance pattern has not changed much in the last 11 years with only one spike in 2002. Resistance to amoxi-clav mostly remained between 31% and 44% with an average of 41% (Figure 3). Trimethoprim sulfamethazole resistance pattern showed increase in resistance, where the average of the first 5 years was 54.8% which increased to average of 77.4% in last 5 years (Figure 4) and the ciprofloxacin resistance pattern also showed increase in resistance with the average of first 5 years being 48.4% which increased to average of 66.4% in last 5 years (Figure 5).

***E. coli* Resistance pattern to common Oral Antibiotics**

	Ampicillin	Amoxi-clav	Ciprofloxacin	TMP-SMX
2002	85%	70%	48%	67%
2003	83%	40%	44%	57%
2004	87%	41%	46%	62%
2005	87%	41%	37%	43%
2006	76%	31%	67%	45%
2007	85%	34%	64%	73%
2008	79%	34%	66%	76%
2009	79%	36%	59%	76%
2010	88%	44%	65%	76%
2011	87%	43%	66%	88%
2012	88%	42%	76%	71%

Fig.1. *E. coli* resistance over 11 year period

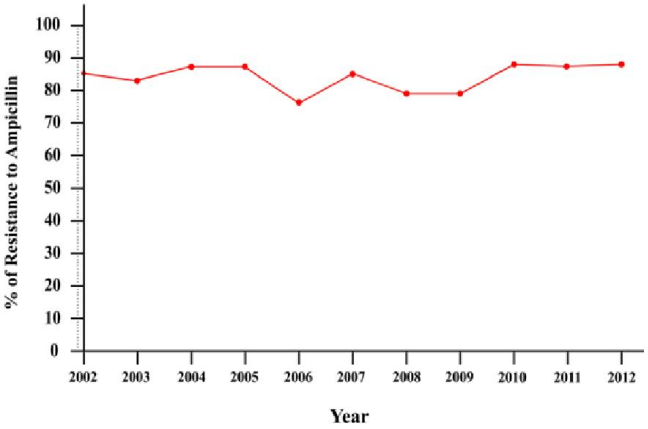

Fig.2. % Resistance of Ampicillin over 11 year's period

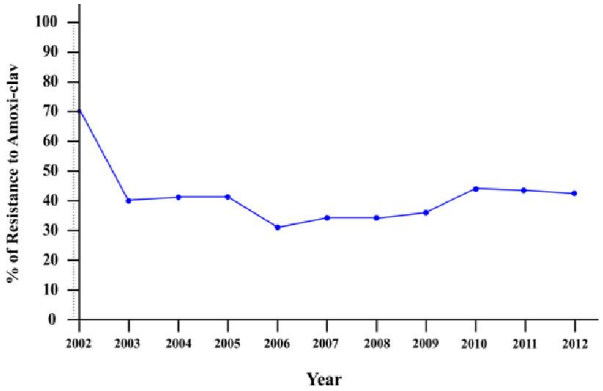

Fig. 3. % resistance of Amoxicillin-clav over 11 year's period

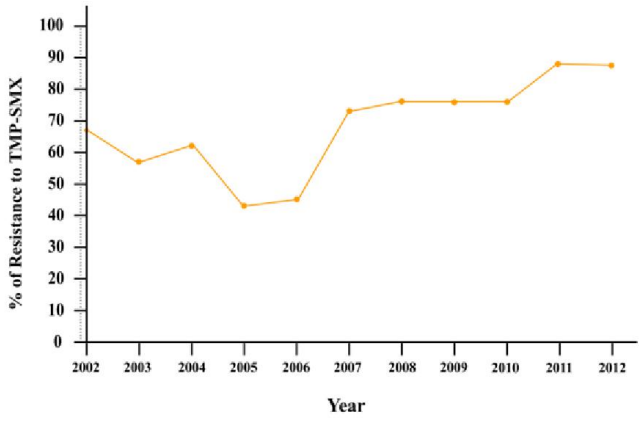

Fig. 4. % Resistance of Trimethoprim Sulfamethazole over 11 year's period

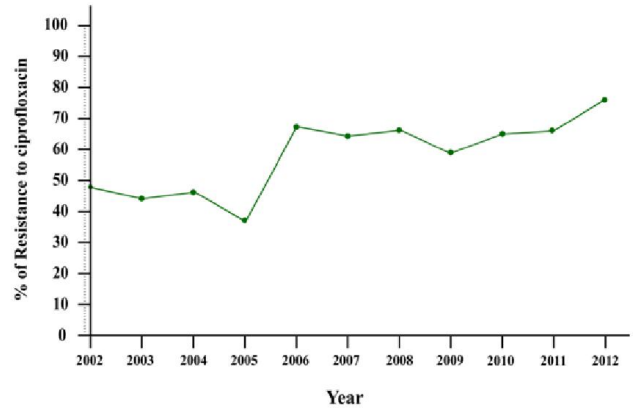

Fig. 5. % resistance of Ciprofloxacin over 11 year's period

One study from Islamabad in 2002 showed resistance toAmpicillin 76%, Trimethoprim Sulfamethazole 72% and ciprofloxacin 49% (Iqbal,). Another study from India showed resistance to Ampicillin (37%), Sulfamethoxazole/ Trimethoprim (29%) and Amoxicillin/Clavulanic acid (29%) (Shakya *et al*., 2013). In the United States Trimethoprim/ Sulfamethoxazole (17.2% to 9.1%), Ciprofloxacin resistance remained low (<1%) (Tadesse *et al*., 1950).Our study shows the resistance pattern of Amoxicillin and Clavulanic acid in past eleven years varying in between 31 to 44 % which is lowest as compared to Trimethoprim, Ampicillin and Ciprofloxacin. Amoxicillin/clavulanic acid is a safe drug with nearly negligible side effects, due to the hydroxyl group present in its structure which makes it compatible to gastrointestinal tract. It is class B drug and so can be safely given in pregnancy. The Clavulanic acid in it, prevents it from the betalactamase enzyme. Resistance is getting common due to plasmid acquisition and amp C enzyme. Amoxicillin plus clavulanic acid is still an effective antibiotic and can be used empirically in treatment of UTI, but measures should be taken to control the developing resistance pattern and decrease the selective pattern.

Conclusion

Our eleventh years study showed a significantly decreasing susceptibility pattern of common oral antibiotics against Escherichia coli. If this trend of resistant continues, the antibiotic is likely to become less effective not only for treating of UTI, but also for treating of other life threating infections. More studies are required from community and hospital settings to determine the resistant pattern of common oral antibiotics. There is a strong need to emphasize on rational and judicious use of common oral antibiotics and adhere to the concept of "reserve drugs" to minimize the misuse of available oral antimicrobials.

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