Proceedings of the World Congress on New Technologies (NewTech 2015)
Barcelona, Spain – July 15 - 17, 2015
Paper No. 155

Ciprofloxacin and Trimethoprim-Sulfamethoxazole Resistance among Bacteria Causing Urinary Tract Infections- A Survey from Pakistan

Tahir Hussain, Saadia Andleeb, Muhsin Jamal
Atta-ur-Rahman School of Applied Biosciences, National University of Sciences and Technology, Islamabad, Pakistan
tahirhussainmdn@gmail.com; saadiaamarwat@yahoo.com; muhsinkhan08@gmail.com;

Maria Zubair
Peshawar Medical and Dental College, Warsak Road Peshawar, Pakistan
mariazubair@live.com;

Abstract **–**Ciprofloxacin and trimethoprim-sulfamethoxazole are the choice drugs for treating urinary tract infections (UTI). Since these two drugs are so frequently used, their overuse has produced generations of resistance bacteria. Antibiotic resistance is an evolutionary process and directly linked to the exposure of bacteria to antimicrobials. The aim of this study was to survey bacterial resistance to these drugs among clinical isolates recovered from urine samples. Seventy three isolates of bacteria were collected from urine samples within a six months period from March to September 2013, in random visits to Microbiology Laboratory, Pakistan Railway General Hospital, Rawalpindi. Bacteria were identified on standard biochemical tests, resistance pattern was determined by Kirby-Bauer disc diffusion methods and interpretation was done according to CLSI guidelines. Most of the bacteria identified were *Escherichia coli* (n=36) followed by *Klebsiella pneumoniae* (n=17), *Proteus vulgaris* (n=9), *Enterobacter aerogenes* (n=7), and *Pseudomonas aeruginosa* (n=4). Trimethoprim-sulfamethoxazole resistance in *E. coli* was (94%), *K. pneumoniae* (88%), *P. vulgaris* (100%), *E. aerogenes* (60%) and *P. aeruginosa* (100%). Ciprofloxacin resistance found among *E. coli* was (89%), *K. pneumoniae* (71%), *P. vulgaris* (40%), *E. aerogenes* (100%), *P. aeruginosa* (100%). This overwhelming level of resistance is extremely alarming because these are the primary drugs administered for controlling urinary tract infections (UTI). These finding suggest that these drugs are no more that effective for treating urinary tract infections. As it takes about a decade to make a new antibiotic available in the market, so there is an urgent need to revise the policy of antibiotic therapy; otherwise their overuse and misuse will make all the antibiotics ineffective and will put the human race at an unavoidable disaster.

Keywords**:** Antibiotics, resistance, ciprofloxacin, trimethoprim-sulfamethoxazole, UTI, Pakistan

1. Introduction

Urinary Tract Infections (UTIs) are globally the most commonly encountered bacterial infections among humans resulting in increased hospitalization and incalculable losses to economy (Ullah et al., 2009). Women are more susceptible to Urinary tract infections than men, with a lifetime risk greater than 50% (Griebling, 2005), and with a slightly lower rate of annual incidence in young women (10%) than old (15%) (Rizvi and Siddique, 2010). Urinary tract infections are commonly caused by *Eschericia coli, Klebsiella pneumoniae, Enterobacter spp*., *Proteus spp*., *Pseudomonas aeruginosa*, *Enterococcus spp*., and *Streptococci spp.* (Akram et al., 2007). However, the most frequently encountered bacteria causing urinary tract infections is *Escherichia coli,* responsible for 75% to 90% of uncomplicated urinary tract infect (Kothari and Sagar, 2008).

Trimethoprim-sulfamethoxazole and ciprofloxacin are the frequently prescribed drugs for urinary tract infections. However, their misuse and overuse has made bacteria resistant and their efficacy has been constantly challenged. Resistance to trimethoprim-sulfamethoxazole was reported first time in

1973(Bushby, 1973), but due to its low cost, it is still used as a front line drug for treating UTI in developing countries (Eliopoulos and Huovinen, 2001). Ciprofloxacin is the second option after trimethoprim-sulfamethoxazole for treating UTI. Since ciprofloxacin are broad spectrum and are also used for variety of infections, an alarming level of resistance has been reported. Various reports have proved the direct correlation between the overuse of antibiotics and resistance in bacteria (Davies and Davies, 2010). The irresponsible practice of medicine positively contributes to this problem as more than 50% of medicines worldwide are misprescribed (Hussain et al., 2014a).

Routine screening of antibiotics administered in a local community is very important not only for proper empirical therapy but also to identify any emerging threats of resistant bacteria. The aim of this study was to survey trimethoprim-sulfamethoxazole and ciprofloxacin resistance among bacteria causing urinary tract infections.

2. Materials and Methods

2. 1. Bacterial samples

Seventy three clinical bacterial isolates were collected from urine samples within a six months period from January to June, 2013 in random visit to Microbiology Lab., Pakistan Railway General Hospital, Rawalpindi. Identification of bacteria was done through standard biochemical tests (Cheesbrough, 2006).

2. 2. Inoculum preparation

Bacteria were grown in Mueller-Hinton broth and turbidity of the culture was adjusted to 0.5 McFarland standards as per CLSI guidelines. These isolate were then swabbed under sterile conditions on Mueller- Hinton agar plates.

2. 3. Disc diffusion tests and interpretation

Discs were placed on Mueller-Hinton agar plates and incubated overnight at 37 degree Celsius. Interpretation of resistance and susceptibility was done according to CLSI guidelines (CLSI, 2013).

3. Results

Most of the bacteria identified were *E. coli* (n=36) followed by *K. pneumoniae* (n=17), *P. vulgaris* (n=9), *E. aerogenes* (n=7), and *P. aeruginosa* (n=4). Trimethoprim-sulfamethoxazole resistance in *E. coli* was (94%), *K. pneumoniae* (88%), *P. vulgaris* (100%), *E. aerogenes* (60%) and *P. aeruginosa* (100%). Ciprofloxacin resistance found among *E. coli* was (89%), *K. pneumoniae* (71%), *P. vulgaris* (40%), *E. aerogenes* (100%), *P. aeruginosa* (100%), Fig. 1.

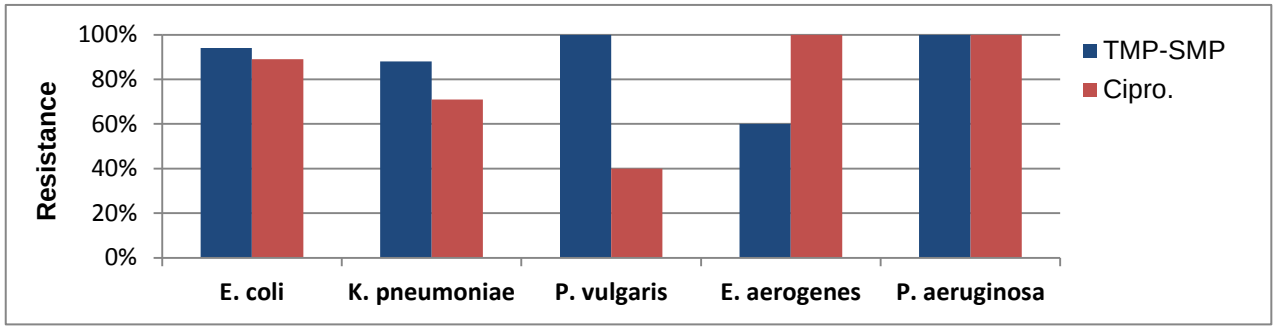

Fig. 1. Resistance profile of bacteria isolated from urine samples.
TMP-SMP: Trimethoprim-sulfamethoxazole,
Cipro: Ciprofloxacin.

4. Discussion

Antibiotic resistance in bacteria is such a complex phenomenon that once bacteria become resistant, this resistance replicates and is shared among diverse bacterial communities (Hussain, 2015). The reason for this replicating resistance is mobile genetic elements like plasmids, transposons, integrons and phages (Huovinen et al., 1995). Once bacteria become resistant to a particular antibiotic, then this resistance can never be removed from bacterial communities (Hussain et al, 2014b; Hussain et al., 2014c). In a study reported on antibiotics resistance (Enne et al., 2001), *Escherichia coli* isolates showed very high level of resistance even when prescription of trimethoprim-sulfamethoxazole was controlled.

Trimethoprim-sulfamethoxazole is used against a wide spectrum of bacteria including pathogens causing urinary tract infections. Trimthoprim-sulfamethoxazole synergistically inhibits folic acid production in bacteria which is required for DNA synthesis. Resistance to trimethoprim-sulfamethoxazole was reported about forty years ago (Bushby, 1973), but it is, still, considered a drug of choice in many developing countries for treating urinary tract infections (Flores-Mireles et al., 2015).

Ciprofloxacin is the second option after trimethoprim-sulfamethoxazole for treating urinary tract infections. Gram negative bacteria, which generally cause urinary tract infections, mainly pose resistance to ciprofloxacin either by mutation in gyrA gene or by presence of qnr genes (Jacoby et al., 2008). Many reports suggest that overuse of ciprofloxacin is responsible for such overwhelming resistance. Overuse of ciprofloxacin not only make bacteria resistant to itself but also induce resistance to several other classes of antibiotics as well (Borgmann, 2012). Resistance to both these important antibiotics is extremely threatening because they are used as front line therapeutic agents in empirical treatment for urinary tract infections. In low income countries where on one hand people have limited access to modern medication, and on other hand hospital staff poorly manages the diseases, in such circumstances resistant bacteria pose the biggest threat to human health.

Recent estimates predict that economic losses incurred due to infections of multidrug resistant (MDR) bacteria are incalculable, but mortality and morbidity rates are increasingly becoming more significant rendering MDR pathogens soon to be the leading cause of deaths worldwide (O'Neil, 2014).

5. Conclusion

Resistance to antimicrobials posed by pathogenic bacteria is a huge threat to human health today. It is a high time for the drugs regulatory bodies to make direct intervention and ensure the proper consumption of antibiotics. Continuous surveillance programs for studying antibiotic resistance in bacteria and consequently revisions in antibiotic policy should be promptly and strictly adapted. Self-medication and over the counter availability of antibiotics must be banned. If this malpractice of antibiotics therapy is not revised, then along with the potential threats of epidemics; super resistant bugs will cause a complete failure of all medical treatments.

Acknowledgements

This study was partially funded by a research grant from the Higher Education Commission, Pakistan. We also thank the pathology lab staff of Pakistan Railway General Hospital, Rawalpindi for their technical support.

References

Akram, M., Shahid, M., & Khan, A.U. (2007). Etiology And Antibiotic Resistance Patterns Of Community-Acquired Urinary Tract Infections In JNMC Hospital Aligarh, India. *Annals of clinical microbiology and antimicrobials,* 6(1), 4.

Borgmann, S. (2012). Ciprofloxacin: One Drug – Numerous Collateral Damages. *Journal of Bacteriology and Parasitology,* 3-6.

Bushby, S.R.M. (1973). Trimethoprim-Sulfamethoxazole: In Vitro Microbiological Aspects. *Journal of Infectious Diseases,* 128(3), S442-S462.

Cheesbrough, M. (2006). *District laboratory practice in tropical countries. Second edition. Part II*. Great Britain: Cambridge University Press. 62-70.

Church, J.A., Fitzgerald, F., Walker, A.S., Gibb, D.M., & Prendergast, A. J. (2015). The Expanding Role of Co-Trimoxazole in Developing Countries. *The Lancet Infectious Diseases.*

Clinical and Laboratory Standards Institute. (2013). Performance Standards for Antimicrobial Susceptibility Testing. *Informational Supplement*, Wayne (PA).

Davies, J., & Davies, D. (2010). Origins and Evolution of Antibiotic Resistance. *Microbiology and Molecular Biology Reviews,* 74(3), 417-433.

Eliopoulos, G. M., & Huovinen, P. (2001). Resistance to Trimethoprim-Sulfamethoxazole. *Clinical Infectious Diseases,* 32(11), 1608-1614.

Enne, V. I., Livermore, D. M., Stephens, P., & Hall, L. M. (2001). Persistence of Sulphonamide Resistance in Escherichia Coli in the UK despite National Prescribing Restriction. *The Lancet,* 357(9265), 1325-1328.

Flores-Mireles, A.L., Walker, J.N., Caparon, M., & Hultgren, S.J. (2015). Urinary Tract Infections: Epidemiology, Mechanisms of Infection and Treatment Options. *Nature Reviews Microbiology,* 13(5), 269-284.

Griebling, T.L. (2005). Urologic Diseases in America Project: Trends in Resource Use for Urinary Tract Infections in Women. *The Journal of Urology,* 173(4), 1281-1287.

Hussain, T. (2015). Pakistan at the Verge of Potential Epidemics by Multi-Drug Resistant Pathogenic Bacteria. *Advancements in life sciences,* 2(2), 46-47.

Hussain, T., Jamal, M., Nighat, F., & Andleeb, S. (2014a). Broad Spectrum Antibiotics and Resistance in Non-target Bacteria: An Example from Tetracycline. *Journal of Pure and Applied Microbiology,* 8(4), 2667-2671.

Hussain, T., Jamal, M., Nighat, F., & Andleeb, S. (2014b). 3rd Generation Cephalosporin Resistance in Klebsiella pneumoniae from Pus Samples. *World Journal of Zoology,* 9(4), 276-280.

Hussain, T., Jamal, M., Nighat, F., & Andleeb, S. (2014c). Susceptibility Profile of Klebsiella Pneumoniae Isolates from Pus to Beta-Lactam/Beta-Lactamase Inhibitor Combinations. *World Journal of Zoology,* 9(2), 115-120.

Huovinen, P., Sundström, L., Swedberg, G., & Sköld, O. (1995). Trimethoprim and Sulfonamide Resistance. *Antimicrobial Agents and Chemotherapy,* 39(2), 279-289.

Jacoby, G., Cattoir, V., Hooper, D., Martínez-Martínez, L., Nordmann, P., Pascual, A., & Wang, M. (2008). Qnr Gene Nomenclature. *Antimicrobial Agents and Chemotherapy,* 52(7), 2297-2299.

Kothari, A., & Sagar, V. (2008). Antibiotic Resistance in Pathogens Causing Community-Acquired Urinary Tract Infections in India: A Multicenter Study. *The Journal of Infection in Developing Countries,* 2(5), 354-358.

Rizvi, R.M., & Siddiqui, K.M. (2010). Recurrent Urinary Tract Infections in Females. *Journal of the Pakistan Medical Association,* 60(1), 55-9.

Ullah, F., Malik, S., & Ahmed, J. (2009). Antibiotic Susceptibility Pattern and ESBL Prevalence in Nosocomial Escherichia Coli from Urinary Tract Infections in Pakistan. *African Journal of Biotechnology,* 8(16), 3921-3926.

Web sites:

Web-1: http://www.jpiamr.eu/wp-content/uploads/2014/12/AMR-Review-Paper-Tackling-a-crisis-for-the-health-and-wealth-of-nations_1-2.pdf., consulted 27 December 2014.