INCREASE IN RESISTANCE AMONG UROPATHOGENS E. COLI TO EMPIRICAL THERAPY

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Received 20/8/2009 Accepted 11/1/2010

ABSTRACT: The objective of this study was to determine the antimicrobial susceptibility among *E. coli* and other uropathogens to trimethoprim-sulfamethoxazole (TMP-SMX), ciprofloxacin and other commonly used antimicrobial agents. This cross-sectional study obtained urine samples from female patients attended Abha National Polyclinic (ANP) and Alanwar Specialized Medical Laboratory (ASML) in Abha city, KSA. Out of 560 urine samples from female patients collected between December 2006 and June 2007, 505 showed significant bacteriuria for various organisms. All Gram negative rods and entercocci were identified using API 20E and API 32 strips respectively. Staphylococci were identified by catalase, coagulase and D'Nase tests. Antimicrobial sensitivity testing of all isolates was performed for 28 various antimicrobial agents on Mueller Hinton agar plates by Kerby Bauer method. The results of the present work indicated that *E. coli* (57.2%) followed by *Klebsiella* spp. (8.1%) and *Enterococcus* spp. (7.3%) are the most common organisms causing UTI in this community. Among *E. coli* isolates high resistance (49.8%) was observed against TMP-SMX which is the first line therapy for community-acquired urinary tract infections (CAUTIs). Moreover, low resistance (6.9%) was recorded against ciprofloxacin which is an alternative treatment. While other selected agents varied in their resistance rates, the incidence of antimicrobial resistance of uropathogenic *E. coli* (UPEC) isolates to: amoxicillin, ampicillin, carbenicillin, ciprofloxacin, nalidixic acid, norfloxacin, novobiocin, ofloxacin, rifampicin, streptomycin, TMP-SMX and sulphonamides were found significantly greater ($p < 0.05$) in adults than in children. The incidence of antimicrobial resistance of UPEC isolates to amoxicillin, ampicillin, carbencillin, cefixime, cefuroxime, ciprofloxacin, nalidixic acid, norfloxacin, ofloxacin, streptomycin, TMP-SMX, sulphonamides, and tetracycline were significantly greater ($p < 0.05$) for married women than for non-married women. It is concluded that *E. coli* is the most common organisms causing UTI in this community. Pattern of antibiotic susceptibility to first line antibiotics is changing notably to TMP-SMX and other common antimicrobial agents. Antimicrobial susceptibility testing of all isolates is crucial for the treatment of UTI.

Key words: *E. coli,* urinary tract infection, empirical therapy, KSA

INTRODUCTION

Escherichia coli causes the vast majority of UTI in both ambulatory and hospital patients. Pathogenic *E. coli* remains a common and troublesome health problem in many different countries all around the world, resulting in a considerable morbidity and expense (Srinivasan *et al.,* 2003).

The prevalence of antibiotic resistance in community-acquired infections is rising. UTIs account for approximately 15% of all antibiotic prescriptions written in the community (Hillier *et al.,* 2002). Resistance among community-acquired *E coli isolates* has evolved with the sequential introduction and widespread use of various antimicrobials over 5

decades of antimicrobial therapy (Gupta et al., 2003). According to the Infectious Diseases Society of America guidelines, first-line treatment of UTIs has traditionally involved a 3-day regimen of trimethoprim-sulfamethoxazole (TMP-SMX) or TMP alone for patients with sulfa allergies in settings where the prevalence of resistance is <10–20% (Nicolle, 2002; Arslan *et al*., 2005). Increasing resistance testing among community-acquired *E. coli* to TMP-SMX worldwide has led to reassessment of the most appropriate empiric therapy for these infections (Nicolle, 2002). Fluoroquinolones including, ciprofloxacin, are suggested as alternative in community in which TMP-SMX resistance associated with uropathogens is ≥10 % to 20%. However, recent studies also have demonstrated an increase in the isolation of fluoroquinolones resistant *E. coli* (Killgore *et al.,* 2004). Resistance to fluoroquinolones has increased markedly since their introduction for UTI treatment (Astal, 2005). Worldwide prevalence of *E. coli* resistant to TMP-SMX varies, ranging between 18% and 50% (Rafay and Nsanze, 2003).

The objective of this study was to determine the resistance rates among uropathogens *E. coli* and other uropathogens to commonly used antimicrobial agents with emphasis on TMP-SMX and ciprofloxacin

MATERIALS AND METHODS

This prospective cross-sectional study was conducted between December 2006 and June 2007 at Abha National Polyclinic and Alanwar Specialized Medical Laboratory in Abha city, Asir region, KSA. 560 female's patients with clinical evidence of community acquired urinary tract infections (CAUTIs) aged between 3 and 72 years were included in the study.

Mid-stream urine samples were collected and processed for physical, biochemical and bacteriological tests. Positive microscopic samples were processed immediately for the semi quantitative count of bacteria by using standard loop method. Sterile calibrated loop delivered 0.001 ml was used to inoculate CLED, blood agar and MacConkey agar plates. All cultured plates were incubated overnight at 37°C and were examined for countable colonies. Culture results were interpreted as being significant and insignificant according to the standard criteria (Aspevall *et al*., 2002).

Isolated bacteria were subjected to microscopic and appropriate biochemical tests for proper identification in a systematic way according to standard methods. Identification of members of the family *Enterobacteriaceae* was confirmed by using Rapid ID 32 E (Biomerieux Marcy-l'Etoile, France).

Antimicrobial susceptibility testing of bacterial isolates was performed by the disk diffusion method in accordance with the National Committee for Clinical Laboratory standards (NCCLS). Isolated colonies were tested on Muller-Hinton agar plate against 28 commonly prescribed antibiotics and chemotherapeutic agents (**Table 1**) by using standard antibiotics disks (Oxoid, Basingstoke and Hampshire, England). *E.coli* ATCC 25922, *Staph. aureus* ATCC 25923 and *Streptococcus faecalis* ATCC 29212 serve as control.

RESULTS

Significant bacterial growth was detected in 505 (90.2%) of the total 560 urine specimens, while yeast cells was detected in 14 (2.5%) urine specimens, insignificant growth was found in 19 (3.4%) urine specimens and there was no growth in 22 (3.9%) urine specimens.

Out of the 505 isolates, 289 (57.2%) were identified as *E. coli* followed by 31 (8.1%) *Klebsiella* spp., 15 (3%) *Proteus mirabilis,* 12(2.4%) *Pseudomonas aeruginosa*, 11 (2.2%) *Citrobacter freundii*, 7.0 (1.3%) *Enterobacter cloacae*, 3.0 (0.6%) *Providencia* spp. and 4.0 (0.8%) were other un-identified gram negative rods.

The results of susceptibility/ resistance of the 289 uropathogenic *E. coli* strains to the 28 antimicrobial agents are shown in **Table 1**.

Table 1. Antimicrobial susceptibility pattern of E. coli isolated from cases of community-acquired urinary tract infections amongst in Asir region

Antibiotics	Sensitive (%)	Resistant
Amikacin	279 (96.5%)	10 (3.5%)
Amoxicillin	98 (33.9%)	191 (66.1%)
Ampicillin	101(34.9%)	188 (65.1%)
Augmentin	266 (92%)	23 (8.0%)
Carbencillin	139 (48.1%)	150 (51.9%)
Cefaclor	264 (91.3%)	25 (8.7%)
Cefixime	267 (92.4%)	22 (7.6%)
Ceftriaxone	276 (95.5%)	13 (4.5%)
Cefotaxime	278 (96.2%)	11 (3.8%)
Cefuroxime	269 (93.1%)	20 (6.9%)

Chloramphenicol	248 (85.8%)	41 (14.2%)
Ciprofloxacin	269 (93.1%)	20 (6.9%)
Erythromycin	19 (6.6%)	270 (93.4%)
Fusidic acid	14 (4.8%)	275 (95.2%)
Gentamicin	281 (97.2%)	8.0 (2.8%)
Methicillin	8.0 (2.8%)	281(97.2%)
Nalidixic acid	193 (66.8%)	96 (33.2%)
Nitrofurantoin	273 (94.5%)	16 (5.5%)
Norfloxacin	269(93.1%)	20 (6.9%)
Novobiocin	23 (8.0%)	266 (92.0%)
Ofloxacin	269 (93.1%)	20 (6.9%)
Rifampicin	178 (61.6%)	110 (38.1%)
Streptomycin	114 (39.4%)	175 (60.6%)
Trimethoprim-sulfamethoxazole	145 (50.2%)	144 (49.8%)
Sulphonamides	119 (41.2%)	170 (58.8%)
Tetracycline	196 (67.8%)	93 (32.2%)
Tobramicin	276 (95.5%)	13 (4.5%)
Vancomycin	12 (4.2%)	277 (85.8%)

DISCUSSION

In this study 505 (90.2%) mid-stream urine samples gave significant growths which were collected from female outpatients diagnosed of having CAUTIs in Aseer region (KSA).

In the present study, *E. coli* predominates among gram-negative bacteria followed by *Klebsiella* spp., *Proteus mirabilis, Pseudomonas aeruginosa*, and *Citrobacter freundii.* Among gram-positive bacteria, *Enterococcus* spp, followed by *Staphylococcus aureus, Staphylococcus saprophyticus* and group B streptococci were the dominant species, similar to many other research conducted in other parts of the world (Tony, 2002; Rafay, and Nsanze, 2003; Uwaezuoke and Ogbulie, 2006).

In the present study, resistant rate to antimicrobial agents among uropathogenic *E. coli* varied (**Table 1)**, it showed high resistant rates which are similar to many reports published by other workers: In the Eastern province of Saudi Arabia as was previously reported increased rate of bacterial resistance to co-trimoxazole (49%), ampicillin (36%), amoxicillin (36%) and tetracycline (52%) (Al-Ghamdi *et al.,* 1999). In Sudan, Ahmed *et al.* (2000), reported high rates of resistance to ampicillin, amoxicillin, co-trimoxazole, tetracycline, sulfonamide, trimethoprim, streptomycin, and carbenicillin among common uropathogenic *E. coli.* In Jordon, Abu Shaqra (2000), reported resistant rates of uropathogens isolates to tobramicin, ceftriaxone and gentamicin as 7%, while the resistance varied from 9-18% for amikacin, ciprofloxacin, norfloxacin, nalidixic acid and cefuroxime.

In this study, highly resistant rate of TMP-SMX among uropathogenic *E. coli* was recorded as 49.1% and among all uropathogenic isolates was 48.2%, this finding is in agreement with reports of other workers as in Saudi Arabia, Jordon and Sultanates Oman, The rate of resistance to TMP-SMX among uropathogenic *E. coli* previously reported as 49%, 48% and 45% respectively (Abu Shaqra, 2000; Al-Ghamdi, 2001; Rafay and Nsanze, 2003). But in Nigeria and Palestine, the rate of resistant was 64.4% and 65.3% respectively. In Sudan, *E. coli* showed high resistance rates against TMP-SMX, similar finding was obtained from Central Republic of Africa (Ahmed *et al.,* 2000 and Lerible *et al.,* 2003).

Ciprofloxacin and other fluoroquinolones are recommended for the empiric UTI treatment in communities in which uropathogens resistance to TMP-SMX was ≥ 10% to 20% (Henry *et al.,* 2002; Killgore *et al.,* 2004). Fluoroquinolones are also considered first-line treatment in patients with pyelonephritis or complicated UTIs. In this study, fluoroquinolones such as ciprofloxacin showed resistant rate to *E. coli* isolates, Three of selected fluoroquinolones ciprofloxacin, ofloxacin and norfloxacin gave same resistant rates for *E. coli* strains (6.9%), On the other hand, resistance rate to nalidixic acid for *E. coli* strains isolated was found very high (33.2%). As in the present study, many other reports have shown increased resistance to fluoroquinolones by various clinical isolates (Perez *et al.,* 1993).

Nitrofurantoin has been used to treat UTIs for over 50 years. It has shown little sign of resistance and is used primarily in patients who require at least seven days of therapy or UTI prophylaxis, as it achieves lower cure rates 85% and serum concentrations than either TMP-SMX or quinolones (Nicolle, 2003). These findings agree with the present results, because low resistant rate among uropathogenic *E. coli* to nitrofurantoin (5.5%) was recorded in this study.

Among β-lactam antibiotics mainly penicillins and cephalosporins groups, the present results demonstrate that uropathogenic *E. coli* isolates have a high level of resistance to methicillin (97.2%), amoxicillin (66.1%), ampicillin (65.1%) and

carbencillin (51.9%). This observed resistance to these drugs is a probable indication of earlier exposure of the isolates to these drugs, which may have enhanced resistant development (Ehinmidu, 2003). However, the long-term usage of penicillins has led to increased resistances to the drug in infections caused by *E. coli* (Lazarevic *et al.,* 1998). But the present study detected low resistant rates to other *ß*-lactams namely: cefaclor (8.7%), augmentin (8.0%), cefixime (7.6%), cefuroxime (6.9%), ceftriaxone (4.5%) and cefotaxime was (3.8%). Similar high resistance rates to penicillins mainly for ampicillin, amoxicillin and carbenicillin were found in previous studies of *E. coli* isolates in Sudan, Saudi Arabia and Nigeria (Ahmed *et al.,* 2000; Akbar, 2001; Ehinmidu, 2003). In France, the antibiotics resistant rates for *E. coli* isolates were 41.3% to amoxicillin. Cephalosporins have been found effective against *E. coli* isolated from CAUTIs (Goldstein, 2000) and similar finding was reported in Spain (Junquera *et al*., 2005).

Aminoglycosides are used parenterally in the hospital setting to treat gram-negative organisms that cause more severe urinary tract infections in acute pyelonephritis and in complicated UTIs (Foster and Marshal, 2004). In the present study, aminoglycosides (gentamicin, amikacin and tobramicin) showed the lowest resistant rate among all selected antibiotics to *E. coli* strains which were: 2.8, 3.5% and 4.5%, respectively. While the resistant rate among *E. coli* strains was high to streptomycin (60.6%) as reported in others studies (Abu Shaqra, 2000).

The present study concluded that *Escherichia coli* accounts for 57.2% of community-acquired urinary tract infections diagnosed in Aseer Region. The resistance rate of uropathogenic *E. coli* to trimethoprim-sulfamethoxazole (TMP-SMX) was found very high (49.8%). Due to the prevalence of *E coli* strains resistant to TMP-SMX, the effectiveness of older therapeutic choices for the treatment of UTIs has become questionable. Resistance rates to fluoroquinolones such as ciprofloxacin among uropathogenic *E. coli* is increasing (6.9%). The most useful antibiotics in this study were: amikacin, gentamicin, tobramicin ciprofloxacin, ofloxacin, norfloxacin, nitrofurantoin, augmentin and second and third generation cephalosporins, because they inhibited uropathogenic *E. coli* and other commonly isolated gram-negative organisms, which constituted 73.9% of the total uropathogens. TMP-SMX, nalidixic acid, tetracycline, sulphonamides, ampicillin, amoxicillin, carbenicillin were found poorly effective *in-vitro* against uropathogenic *E. coli* and to the majority of the other isolated organisms, thus could not be useful for treating CAUTIs in our study area. Erythromycin, fusidic acid, novobiocin, methicillin and chloramphenicol were found poorly effective against uropathogenic *E. coli* but may be useful for gram-positive cocci infections. Epidemiological and patients factors namely: recurrent of infections, marital status, age and diabetes accelerate the resistance rate. Continued surveillance of resistance rates among uropathogens is needed to ensure appropriate recommendations for the empiric therapy for UTIs.

ACKNOWLEDGEMENTS: It is a pleasure to thank the staff of Abha National Polyclinic, special thanks to Dr Ehab Mousa and Mrs. Mini Ninan and to Dr Ahmed Hamid Kisha and Mr. Mohamed Alrshed from Alanwar Specialist Laboratory.

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