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PREVELANCE OF UTIS AND ASSESSMENT OF THE ANTIBACTERIAL ACTIVITY OF ANTIBIOTICS AND HERBAL EXTRACTS ON MDR UROPATHOGENS

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Abstract
Introduction-Bacterial urinary tract infections (UTIs) in humans are the rising challenges worldwide especially the emergence of the multidrug resistant (MDR) bacterial strains. Individuas of ages may be affected by simple and complex form of UTIs. These may be caused by both gram negative and gram positive bacteria. The MDR uropathogens are recognized for causing complex UTIs worldwide.
Materials and method-The focus of this study is on the frequency of UTIs caused by MDR gram negative bacteria and the antibacterial activity of antibiotic and their susceptibility pattern to antibiotics and herbal extracts (*Oregano vulgare* L.*,* and *Thymus vulgaris* L. A total of 1128 (n=1128) urine samples were collected from 5–25, 26–50, and 51–75 years of patients of both genders. The samples were inoculated media for microbial growth, characterization, and susceptibility of test antibiotics and herbal extracts.
Result-Our findings revealed that females are more affected than males, with 63%, 63%, and 59% when examined the catheterized, midstream and diabetic urine samples predominantly caused by *E. coli* followed by *K. pneumoniae* 15%, 17%, 15%; *P. mirabilis* 14%, 14.5%, 14%; *E. cloacae* 5%, 2%, 1.5%, and *P. aeruginosa* 2%, 3%, and 10% in both genders, respectively.
Conclusion-Antibiotic susceptibility revealed the greater resistance of *E. coli* to amoxicillin-clavulanate, cefotaxime, amoxicillin-clavulanate, nitrofurantoin, and ciprofloxacin followed by *K. pneumoniae*, *P. mirabilis*, *Enterobacter cloacae*, and *P. aeruginosa*. The synergistic effect of both extracts showed a more significant influence at concentrations of 1.0, 1.0, 1.2, 1.2, and 1.0 mL/100 mL. respectively. The antibacterial properties of *Oregano vulgare L.* and *Thymus vulgaris L.* exhibited increased effectiveness at concentrations of 1.2, 1.4, 1.2, 1.2, and 1.2, respectively.

Keywords: UTI, Uropathogens, MDR Strains, Antibacterial Effects.

1. Introduction

UTIs occur in all ages, especially females from adolescence to old age.(1-2) There are two types of UTIs namely the uncomplicated, which occurs in non-pregnant adult women and complicated arises when the infection poses an increased risk of complications, frequently as a result of pre-existing health issues.(13) *E. coli* is major UTI causing pathogen called as uropathogenic *E. coli* (UPEC),(4-10), which belongs to family Enterobacteriaceae.(11). While UTIs are known to be mild and treatable but could be chronic if untreated causing renal damage. Pyelonephritis is certainly a major abnormal complication during pregnancy for both the pregnant female and new born baby. It ranks 2-4% of pregnancies, which may be related to asymptomatic bacteriuria among the 2-7 patients.[12-13] About 10% of pregnant females are known to suffer from UTIs due to the invasion of the urethra through the bladder by bacterial populations from the gastrointestinal tract and occurs during travel.[14-15] UTIs frequently cause anxiety and sickness in females.[14] Women are more susceptible to complex UTIs that escalate to serious conditions such as pyelonephritis or urosepsis. Bactriuria in female patients may rise during pregnancy that leads to greater death rates, low-birth weight and prematurity. [16-17] MDR uropathogens are recognized for causing complex urinary tract infections (UTIs), especially in individuals with urolithiasis, neurogenic bladder, indwelling catheters, renal transplants, and immunosuppression. These conditions promote the invasion and retention of uropathogens within the urinary tract.[18]

Several studies on antibiotic susceptibility testing of uropathogens [19-22] have been reported in Pakistan, but no true cure has yet been explored. This study aimed to determine the prevalence of UTIs in both genders of different age groups in the rural area of Jamshoro, to identify the major uropathogens by culturing technique and their antibacterial activities and to identify the synergistic effects of *Oregano vulgare L.,* and *Thymus vulgaris L.* as alternate medicine.

2. Materials and methods

Patients aged 5–25, 26–50, and 51–75 years of both genders have been diagnosed. A survey form was filled out with the name, age, sex, geographic distribution, occupation, and family history, duration of the hospitalization, previous antibiotic therapy, and laboratory diagnosis.[23]

2.1. Sample Size

A total of 1128 (n=1128) catheterized, midstream and diabetic urine samples were collected separately from the OPD of private clinics and hospitals. Equal numbers of urine samples n=564 from each gender were collected from the patients of age groups 5-25 (n=216), 26-50 (n=268), 51-75 (n=80) from males, and 5-25 (n=236), 26-50 (n=276), 51-75 (n=52) from females.

2.2. Inoculation and Incubation

Urine samples were separately inoculated onto the surface of sterile MacConkey's agar, and Cystine-lactose Electrolyte Deficiency agar plates (Oxoid-UK), a disc of ceftazidime antibiotic (2 mg/L) was placed over the surface of inoculated plates, and incubated at 37°C overnight. A bacterial suspension (10^5cfu/mL) was prepared after incubation for future processing in colony, microscopy, and biochemical analyses.[16, 25]

2.3 Determination of antibacterial activity

Assessing the susceptibility of clinical isolates to antimicrobials as agar diffusion methods was applied.[26]. Antibiotic discs / µg of ampicillin 10, amoxicillin 25, amoxicillin/clavulanate 30, aztreonam 30, cefotaxime 30, ceftazidime 30 ceftriaxone 30, ciprofloxacin 5, gentamycin 10, Norfloxacin 10 and Nitrofurantoin 300 µg.[27-28].

The herbal extracts of *Origano vulgare L.,* and *Thymus vulgaris* L. leaves were cleaned with tap water, afterward peeled and dried in dim at room temperature for 24 hours, later ground to powder, and was kept in dry containers independently.[29] An equal quantity of both powdered extract was blended in sterile distilled water and 20 g of the blend was arranged by macerating in 100 ml in a sterile bottle and cleared out at room temperature in orbital shaker for 24 hours and later sieved.[30]. The fluid extricates (10 mL) once more centrifuged at 1500 rpm for 15 minutes and kept at 50ºC for 2 h., later cooled and sieved by Whatman filter paper,[31] and finally stored at room temperature for 24 hours. The alkaloids have been identified from leaves of both herbs from a few milliliters of aqueous separately filtered extract by pouring a drop of Dragendorff reagent by the side of the test tube. Later a magnesium powder and a few drops of strong hydrochloric acid were placed in the test tubes to ensure that both extracts contained flavonoids.[27, 32]

2.3.1 Disc diffusion method

The test cultures were prepared in a sterile test tube, achieving a concentration of 10^5 cfu/mL. Using soaked Whatman filter paper (13 mm) discs of 9 mm in each dilution of extract (v/v) for 10 seconds were placed over a series of Mueller Hinton Agar (MHA) plates that contained the test inoculum.[(33)] To observe the colonies, the plates were incubated at 37°C for 24 hours containing 5% Dimethylsulfoxide (DMSO) served as the negative control, and a 30 µg amoxicillin-clavulanate disc was placed as a positive control.[(34)]

2.3.2 Well diffusion method

Five wells of 9 mm diameter were designed using a series of MHA plates, each composed of different test isolates. One microliter (µL) of melted nutritional agar was put into each well, and then it was let to set for 20 minutes. Both herbal extracts were added to each well of the plate containing 0.2, 0.4 to 1.4 mL of test extract with 5% DMSO as a negative control, and clavulanate amoxicillin discs as a positive control. The plates were then left at room temperature for 2 hours before being incubated for 24 hours at 37°C.[(30)] Following CLSI guidelines, the ATCC cultures were used. *E. coli* (ATCC 25922), *K. pneumoniae* (ATCC 70063), *P. mirabilis* (ATCC 25933), *E. cloacae* (ATCC 13047), and *P. aeruginosa* (ATCC 275833) as standards, revealed inhibitory zone in different sizes in comparison to the CLSI standard zones. [(27-28)]

3. Results

The present work is conducted to determine the frequency of UTIs caused by uropathogens in rural areas of Jamshoro. Urine samples were collected from symptomatic patients of test age groups of both genders. A total of 216, 268, 80, and 236, 276, 52 catheterized, midstream, and diabetic urine samples were collected from male and female patients respectively (Table 1). Isolated samples underwent culturing and characterization, leading to notable results that include *E. coli* (64%), (60%), (61%); *K. pneumoniae* n=31 (14%), n=49 (18%), n=12 (15%); *P. mirabilis* n=32 (15%), n=46 (17%), n=11 (14%); *E. cloacae* n=12 (5.5%); n=5 (2%); n=1 (1%); *P. aeruginosa* n=5 (2%); n=6 (2%); n=7 (9%) whereas in female patients *E. coli* n=151 (64%); n=182 (65%), n=30 (58%); *K. pneumoniae* n=35(15%), n=44 (16%), n=8 15%); *P. mirabilis* n=33 (14%), n-33 (12%), n=07 (13.5%); *E. cloacae* n=12 (5%), n=08 (3%), n=01 (2%); *P. aeruginosa* n=05 (2%), n=09 (3%); n=06 (11.5%) in catheterized, midstream and diabetic urine samples of 5-25, 26-50 and 51-75 years of age groups respectively (Table 2, 3, Figure 1).

The findings of clinical isolates in all age groups of both genders revealed total numbers of *E. coli* n=708 (63%, 63%, 59%), *K. pneumoniae* n= 179 (15%, 17%, 15%), *P. mirabilis* n=163 (14%, 14.5%, 14%), *E. cloacae* n=40 (5%, 2%, 1.5%) *and P. aeruginosa* n=38 (2%, 3%, 10%) (Table 4, Figure 2). *E. coli* 51(7%), 68 (9.5%), 75 (10.5%), 85 (10%), 75 (10.5%), 85 (8%), 59 (10%), 74 (10%), 49 (7%), 59 (8%), 42 (6%); *K. pneumonia* 12 (7%), 25 (14%), 31 (17%), 23 (13%), 21 (12%), 22 (12%), 24 (13%) 16 (9%), 23 (13%). *P. mirabilis* (4%), 24 (13%), 25 (15%), 10 (6%), 11 (7%), 11 (7%), 9 (5%), 21 (13%), 14 (8.5%), 12 (7%), 22 (13%), *E. cloacae* 3 (7%), 2 (5%), 4 (10%),, 4 (10%), 2 (5%), 1 (2%), 3 (12%), 5 (12%), 4 (10%), 6 (15%), 9 (22%), *P. aeruginosa* 0 (0%), 3 (9%), 5 (16%), 2 (6%), 1 (3%), 4 (12,5%), 2 (6%), 7 (22%), 5 (16%), 2 (6%), 2 (6%) against ampicillin, amoxicillin, amoxicillin-clavulanate, aztreonum, cefotaxime, ceftazidime, ceftriaxone, ciprofloxacin, gentamycin, norfloxacin, and nitrofurantoin respectively (Table 5).

The antibacterial activity of *Oregano vulgare L., and Thymus vulgaris l.* was determined by disc diffusion and well diffusion method. Comparative analysis revealed maximum sensitive zones (mm) by the well diffusion methods. The findings revealed 23.1 (1.0), 23.5 (1.2), 21 (1.4), 21.5 (1.2), 24.2 (1.2) (Table 6) and 22.7 (1.2), 20.4 (1.4), 20 (1.2), 22.4 (1.2), 23.8 (1.2) against *E. coli, K. pneumonia, P. mirabilis, E. cloacae, P. aeruginosa* (Table 7). The synergetic effects of both extracts by well diffusion methods showed greater sensitivity zones 25 (1.0), 24.2 (1.0), 21.6 (1.0), 24.3 (1.2), 24 (1.0) against *E. coli; K. pneumoniae; P. mirabilis; E. cloacae; P. aeruginosa* respectively (Table 8).

Discussion

Urinary tract infections pose a significant clinical and economic impacts in both community and hospital settings with higher mortality rates. [35] The present study deals with UTIs in the rural populations of Jamshoro, as observed among different age categories of patients.[35-36] The catheterized, midstream, urine revealed 23%, 55%, 22%, 27%, 56%, and 16% among the test age groups respectively. The discrepancies in urine samples may be due to the environmental influences, socioeconomic conditions, and the demographic attributes of various areas. *E. coli* 60-66% were discovered as the major pathogen followed by *K. pneumoniae* 14-16%, *P. mirabilis* 12-17%, *E. cloacae* 1-5.5%, and *P. aeruginosa* 2-11.5% in UTIs in agreement of [37-39].

K. pneumoniae is the second leading cause, predominantly spread through interpersonal contact, indwelling catheters; substance abusers face an increased risk of infection in hospitals.[40-41] In catheterized patients, *P. mirabilis* infections can arise from mannose-associated fimbriae, and non-agglutinating fimbriae. Furthermore, the autotransporters TaaP and AipA may interact with collagen I and laminin. Hemolysin (HpmA) and the toxic protein agglutinin (Pta) are also important factors that lead to the lysis of host cells, thereby causing UTIs.[39]. *Pseudomonas aeruginosa* is a significant contributor to UTIs among the age group of 51-75 years may be due to its ability to develop multiple drug resistance.[42, 39]. It causes co-infections, and form biofilms on catheters through the production of extracellular polysaccharides through quorum sensing, which triggers the release of extracellular DNA (eDNA), along with an increased release of eDNA from PAO1 strains (ΔlasI, ΔrhlI, and ΔpqsA mutants). [42-43] *E. coli* as the main pathogen causing 66% in females aged 26 to 50, compared to 64% in males. For the 5 to 25 age group, both males and females show a prevalence of 63%, while females aged 51 to 75 have a UTI rate of 59%. This data is consistent with the findings reported in [44]. The UTIs in females may be due to the anatomical structure of the urogenital tract, shorter urethra, pregnancy, recent sexual activity, and the use of spermicide-containing devices. These findings align with previous research.[44-48]

A group of 26-50 years possess UTIs followed by those aged 5-25 and 51-75 due to the effects of estrogen depletion, which leads to vaginal atrophy and labial enlargement.[15-16] This facilitates the pathogens from the vagina to the urethra, fluctuating pH levels, diminished vaginal flora.[17-19] In women, UPEC strains isolated from sexually active individuals.[49] This happens in cases of recurrent UTIs during pregnancy.[45] Additionally, anterior vaginal wall prolapse, and inability to spontaneously void and increased post-void residual volume may contribute UTIs [50]. In men, greater UTI occurs by use of illegal drugs that increases bacterial effects of prostate fluid.[45-46, 51] Our findings were 11.2% of UTIs among diabetic patients. This indicates that diabetic women have a higher infection rate compared to men, mainly due to the age factor, micro-albuminuria concentration, and to a certain degree, glycated hemoglobin. These results are consistent with previous research.[52-53]

All tested bacterial pathogens demonstrated varying levels of resistance to the antibiotics evaluated. [34-35] Our findings revealed significant resistance zones (mm) for *E. coli,* with values of 18 for ampicillin and 17 for nitrofurantoin. *K. pneumoniae* showed resistance zones of 18 for amoxicillin and 16.5 for amoxicillin-clavulanate. *P. mirabilis* exhibited resistance of 18 for both ceftazidime and norfloxacin, and 17 for amoxicillin. *E. cloacae* had resistance zones of 18 for norfloxacin and 17 for aztreonam, ceftriaxone, and gentamicin. *P. aeruginosa* showed resistance of 16 for ceftazidime and ciprofloxacin, and 15 for gentamicin, along with other tested antibiotics. Our observations of MDR align with,[46, 35, 37] which suggest that Gram-negative pathogens may acquire genes responsible for producing ESBL, OXA, AmpC, Oxas, and CTX-M type enzymes.[39]

The insert consists of the integrase gene attI, a transposon, and an integran. It encompasses genes such as ParC and parE, which are responsible for coding topoisomerase IV, in addition to gyrA and gyrB. Notable mutations are identified in parC (S80), gyrA (S83), and gyrB (S464), alongside various beta-lactamase genes including blaTEM, blaTEM-2, blaCMY, blaCTX, blaOXA-1, blaCTX-M, and sul1. Furthermore, it encodes integrin gene cassettes (aadA1, aadA2) that synthesize aminoglycoside adenyltransferases, as well as B, which encodes aminoglycoside-(2)-transferases. The aac (6)-Ib gene is responsible for encoding an aminoglycoside acetyltransferase, while sat2 encodes streptothricin acetyltransferase. Additional genes referenced include rpoB, tufB, rpsL, fusA, and rpoA. The mutations associated with Tumor Endothelial Marker-187, VEB-1, Integrin PER-1, and VIM-1 SHV-type β-Lactamase (38-45) are also noted, along with SGL-1 (SGE-1) and the production of carbapenemase.[50-57] In UPEC, mutations that impede drug uptake are correlated with the deletion of membrane-bound porins and bla genes located on plasmids.[49] The emergence of MDR Gram-ve bacteria is linked to the production of extended-spectrum beta-lactamase enzymes, resulting in prolonged hospitalizations, increased reliance on intravenous devices, and reduced immunity in diabetic patients, catheters-complications, biofilm formation, racial disparities, and antibiotic overuse.[58, 54]

In *Klebsiella pneumoniae*, antibiotic resistance can develop due to a variety of resistance genes, mobile genetic elements like *K. pneumoniae* carbapenemase and New Delhi metallo-ß-lactamase-1, alterations in drug targets, and the expulsion of drugs through ATP-binding cassette transporters. The CTX-M gene and the 16S rRNA methylase RmtA are particularly important in this context. Additionally, the qnrD genes are located on small, non-conjugative plasmids of 2.7 kb and 4.2 kb, which can be obtained via the mobile insertion cassette (Mic) element from *Pseudomonas aeruginosa* revealed a resistance rate of 22% to ciprofloxacin, followed by 16% to both amoxicillin-clavulanate and gentamicin, and 12.5% to ceftazidime, among other antibiotics. These results are consistent with earlier studies,[48, 59], likely due to the production of ESBL and carbapenemase enzymes, along with other contributing factors.[60, 45, 55-56, 61]

Uropathogenic strains of *E. cloacae* are distributed pertaining to the genetic clusters of the complex.[61] *E. cloacae* is common in hospital environments. The development of MDR in *E. cloacae* are associated to the AcrAB-TolC system 'TolC', OqXAB (OqxAB), EmrE, MdfA, MacA, Mar, Ram, Sox, inconsistent antibiotic treatment, and the acquisition and expression of resistance genes such as QnrA, qnrS, blaShV, and blaCX-M. These elements facilitate biofilm formation, corroborating findings from earlier studies.[12, 62] Additionally, *E. cloacae* features complex gene clusters,[62] such as clusters III, VI, and VII, which are associated with two genetically distinct clades: clade 1 and a CGH-based clade 2 related to the hsp60 gene.[60]

Conclusion

District Jamshoro is situated in the western part of the Sindh province, consisting of a rural and urban population. Mostly the poor community with malnutrition and unhygienic standards are more prone to the UTIs. In this study, *E. coli*, *K. pneumoniae*; *P. mirabilis*; *E. cloacae*; *P. aeruginosa* were higher and lower resistance to the respective test antibiotics was observed. When tested, the antibacterial effects of *Oanum vulgare* L., and *Thymus vulgaris* L. the results showed greater effect on the isolates in combination of both extracts. In order to avoid the illegal use of home remedies and antibiotics, the relevant health authorities should initiate awareness programs to implement new policies to deal with the crisis and make recommendations to focus on the production of herbal medicines to support the rural population because of high costs, side effects of antibiotics and the emergence of resistant strains.

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