ejpmr, 2016,3(8), 353-358

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

A SURVEILLANCE STUDY ON THE HUMAN BACTERIAL UROPATHOGENS AND THEIR ANTIBIOGRAM

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Article Received on 16/06/2016 **Article Revised on 30/06/2016** **Article Accepted on 09/07/2016**

ABSTRACT

The aim of this research was to reduce (or) completely eliminate the acute and chronic Urinary tract infections in humans cause by 5 different uropathogens by developing the several antibiotic susceptibility pattern from January 2010 to October 2010, among 50 urine specimens, *E.coli* had the highest frequency in isolator, with the frequency of 25 (50%) followed by *K.pneumoniae* 10 (20%), *Cirtobacter* species 9 (18%), *Enterobacter* species 4 (8%), *Staphylococcus aureus* 2 (4%), *E.coli* exhibited lowest resistant pattern sto almost of all antibiotics and *Enterobacter* exhibited highest resistant pattern.

KEYWORD: *K.pneumoniae, Cirtobacter, Enterobacter.*

1.0 INTRODUCTION

Urinary tract infections are a serious health problem affecting millions of people each year Infections of the urinary tract are the second most common type of infection in the body women are especially prone to UTI's Normally arine is sterile. It is usually free from organisms An infection occurs when tiny organisms, usually bacteria from the digestive tract, cling to the opening of the urethra and begin to multiply. Bacteria multiply in urethra called urethritis if multiply in bladder called cystitis and if multiply in kidney called pyclonephritis. Both men and women, immune defenses also prevent infection but despite the safeguards, infections still occur. People with diabetes have a higher risk of a UTI because of changes in the immune system. Any other disorder that suppresses the immune system raises the risk of a urinary tract infection.

Urinary tract infection is the most common bacterial infection that occurs in older populations. Urinary tract infection in older men and women is usually asymptomatic and accompanied by pyuria. To isolate the bacterial Uropathogens present in the urine sample of patients suffering from acute (or) chronic UTI using standard isolation technique. The majority of community–acquired symptomatic UTI's in elderly woman are caused by E.Coli. The most organisms isolated in children with uncomplicated UTI are Enterobacteriaceae (Akinkugbe FM etal 1973). Among patients with diabetes include klebsiella species, Group B streptococci and enterococcus species as well as E.coli. Patients with spinal cord injuries commonly have E.coli infections na dother common uropathogens include pseudomonas and proteus mirabilis. Citrobocter diversus strains were resistant to 8uglml ampicillin (97.5%) and 32 mug/ml carbenicillin (87.5%) and susceptible to 8 mug/ml cephalosporis. Citrobacter freundii strains were moderately susceptible to 8 mug/ml ampicillin (25%) and susceptible to 8 mug/ml carbenicillin (92%) and resistant to cephalosporin (southern P.MJr etal 1977) Urinary isolates of E.coli klebsieela aerogens and proteus mirabilis were susceptible to nitrofurantoin and to oral cephalsoporins (Alon.U 1987) Nearly half of community UTI infections in women that were caused by E.coli strains with resitance to trimethoprim – sulfomethoxazole (Johnson J.R 1991). UTI was caused mainly by Grano – negative organisms than gram – positive organisms (Akinyeni K.O.etal 1997).

Urinary isolates of E.coli that has been tested against ampicillin, Ciprofloxacin, Nitrofurantoin and Trimethoprim – Sulfomethoxole of those 7.1% were resistant to three (or) more agents and considered multidrug resistant (Daniel F.Sahm etal 2001) E.coli isolates to ampicillin, ciprofloxacin and Nitrofurantoin were slightly varied ciprofloxacin was the only agent studied that demonstrated a consistent stepurise increase in resistance from 1995-2001 (James A.Karlowsky etal 2002) Urinary tract infectins remain the common infections diagnosed in outpatients as well as in Hospitalized patients (Karowsky 1989) current knowledge is mandatory for appropriate therapy. Extended Spectrum beta lactamases. [ESBL] Hydrolysed expanded Spectrum Cephalosporins like Ceftazidime, Cephotaxime which are used in the treatment of UTI.

Producing bacteria May not be detectable by Soutine disk diffusion Susceptibility test, leading to inappropriate Use of antibiotics and treatment failure E.colab, Klebsiella pneumonia and Acinetobacter Were ESBL producing species Multidrug resistance were found to be significantly more in ESBL producing isolates (90.5%) than non ESBL producers (68.9%) (supriya S.Tankhiwale etal 2003).

Klebsiella pneumonia isolates were obtained from sputum, wound swabs and urine and screened for their antibiogram using standard procedures. All the isolates shared multidrug resistance to amoxicuillin and trimethoprim. However the isolates showed marked susceptibilities to norfloxacin (90.01%) Cefuroxime (95.4%) and ciprofloxacin (86.36%) the study has revealed that klebsiella pneumonia isolates are multi-drug reisitant (Titaniji V.Petal (2003) 400 strains of klebsiellae identified by cultural characteristics and bio-chemical reactions were subjected to bio typing and anitbiogram. Based on indole production, pectin and gelatin riquefaction, maximum sensitivity was shown to amikacin (7.2.1%) and maximum resistance to apicillin (87.5%) (Aggarwal etal 2008) Pseudomonas aeruginosa and citrobacter species were the most prevalent isolates, 95.5% of citrobacter isolates were citrobacter freundii isolated from 258 Urine sample (Knorasani.G etal 2009).

2.1. MATERIALS AND METHODS

2.2. Sample collection

Urine sample were collected from in and out patients of the hospital suffering from acute (or) chronic UTI patients samples were collected from patients by mid-stream clean catch procedure. The mid-stream urine was collected from the patients using sterile, day wide – necked leak proff container and Boric acid was added to the urine as it has to be transported to the laboratory. The glassware were washed thoroughly in running top water. rinsed in distilled water and dried in hot air over (max temp 200ºC) Media were sterilized in a portable autoclave at 121ºC and at 15 1bs pressure for 15 minutes. All the inoculation works were carried out under aseptic condition in an Laminar air flow chamber and then incubated at 37º for 24 hours.

2.3. Morphological characterization.

2.4. Gram Staining

The sample was smeared on the slide and heat fixed. The crystal violet dye was added and allowed to cover the whole smear to act for l minute and rinsed with tap eater. Few drops of Grams – iodine was added and allowed to react fot 30 seconds to l minute. It was decolorized with 95% ethanol and immediately washed under running tap water. Safranine was added and allowed to act for l minute, slide was rinsed with tap water and blot dried and examined under oil immersion objective. Gram positive cells were appeared in purple color and gram negative cells were appeared in red color.

2.5. Hanging Drop Method

The overnight broth culture was placed on the center of the cover slip, The cavity slide was placed on the cover slip and turned over to prepare a hanging drop. The slide was viewed under the microscope at 40X. The motility was detected from the movement of the organism.

2.6. Cultural characterisation

2.7. On Nutrient Agar

Nutrient agar was prepared and sterilized at 121º C for 15 minutes at 15 lbs pressure, the sterile medium was poured into the sterile petriplates, the culture was streaked on the paltes,

2.8. On Mac.Conkey Agar

Mac. Conkey agar was prepared and sterilized at 121º C for 15 minutes at 15 lbs pressure, the sterile medium was poured into the sterile petri plates and the culture was streaked on the plates. The formation of pink color colonies indicates the lactose fermentors whereas colorless indicates non lactose fermentors.

2.9. Biochemical characterization

2.10. Indole production test

Peptone broth was prepared and sterilized at 121º C for minutes at 15 lbs pressure. The culture was inoculated into the peptone broth under aseptic condition and incubation at 37º C for 24 hours. Few drops of kovac's reagent were added to the tube after incubation. The formation of cherry red colored ring in the upper surface of the medium indicates positive results.

2.11. Methyl red test

MR- VP broth was prepared and sterilized at 121ºC for 15 minutes at 15 lbs pressure. The culture was inoculated into the broth under aseptic condition and incubated at 37ºC for 24 hours. After incubation, the indicator methyl red was addred to the tubes. Development of red colour indicates acid production and positive result.

2.12. Voges proskauer test

MR-VP broth was prepared and sterilized at 121ºC for 15 minutes at 15 lbs pressure. The culture was inoculared into the broth under aseper condition and incubated at 37º C for 24 hours. After incubation the indicator Barritt' S reagent I and 2 were added. The development of pink colour indicates the positive result.

2.13. Citrate utilization test

Simmon' S citrate agar was prepared and sterilized at 121º C for 15 minutes at 15 lbs pressure. The medium was poured into tube and slant was prepared. The culture was streaked in the slant. It was incubated at 37º C for 24 Hours. Development of blue colored slant after incubation indicates the positive result.

2.14. Urease test

Christenson' s urea agar slants were prepared and sterilized at 121º C for 15 minutes at 15 lbs pressure. The medium was poured into the tube and slant was prepared.

The culture was streaked in the slant. It was incubated at 37º C and the results are recorded after 4,8,12 and 48 hours of incubation. Development of deep pink colored slant after incubation indicates the positive result.

2.15. Triple Sugar Ion test

Agar slants were prepared and sterilized at 121º C for 15 minute at s 15 lbs pressure. The medium was poured into the tube and slant was prepared. The culture was streaked in the slant. Culture ferments lactose and sucrose due their presence in higher concentration, they serves as a substance for continous fermentation and maintain acid reaction in both slant and butt A/A G-A. change of colour from red to yellow was seen in both slant and butt which in turn indicates gas production whereas there was no carbohydrate fermentation occurred and does not show any characteristic change and was found to be negative.

2.16. Catalase test

Agar slants were prepared and sterilized at 121º C for 15 minutes at 15 lbs pressure. The medium was poured into the tube and slant was prepared. The culture was streaked in the slant. After incubation add l ml of 3% H2O2 into the slant. caralase positive culture will produce bubbles of oxygen within l minute after the addition of H2O2 .Release of free O2 gas bubbles was observed as positive. Culture thus showing catalase positive test and release of no bubbles indicates negative catalase test.

2.17. Identitification of the isolates

The isolates were identified according to the standard procedures from simple staining to IMVIC test followed by their confirmatory test like growth in the specific media. The Dominant E.coli isolate were also identified by using molecular technique. For molecular identification, amplified PCR products were separated by Agarose gel electrophoresis and purification of the PCR products is done by Qiaquick PCR purification method. Purified PCR products were sequenced using big dye terminator method. Then the isolates were selected for processing the further steps in the experiment.

2.18. Antibiotic susceptibility testing

Sensitivity Percentage of the isolated organisms were detected by Kirby – Bauer disc diffusion method. Muller – Hinton agar plates was prepared and sterilized and sterile medium was powered into the sterile petriplates Five colonies of the test organisms were sweaked on agar plates using sterile sevaks and the appropriate antibiotic disc's were placed. The plates were than incubated at 37ºC for 18-24 hours. Interpretation of results was done by measuring the zone diameter. Zone of inhiloition was greater than 10mm were considered sensitive, 5mm to 10mm as moderately sensitive and no zone (or) less than 5mm were considered as resistant.

3.0. RESULTS AND DISCUSSION

The selected five isolates were identified by the routine morphological and biochemical tests and the results are represented as follows.

Among 50 urine samples UTI was caused primarily by gram – negative organism 48 (96%) than gram positive organisms 2 (4%) (Table1).

TABLE 1: FREQUENCY OF THE ORGAISMS IN TYPE RELATION TO SEX

TYPE OF ORGANISM	SEX		TOTAL PERCENTAGE
	MALE	FEMALE	
GNB	14	34	48 (96%)
GPC	0	02	02 (4%)

GNB – Gram Negative Bacilli, GPC – Gram Positive Bacilli

Among the isolates, *E.coli* had the highest frequency in isolation with the frequency of 25 (50%) followed by *Klebsilla pueumoniae* 10 (20%) *Citrobacter species* 9 (8%) *Enterobacter species* 4 (8%) and *Staphylococcus aureus* 2 (4%) (Table 2).

TABLE 2: FREQUENCY OF INFECTION IN 50 SAMPLES

S.NO	ORGANISMS	TOTAL NUMBER OF ISOLATES	PERCENTAGE OF INFECTION
1	*Escherichia coli*	25	50%
2	*Klebsiella pneumonia*	1	20%
3	*Citrobacter species*	9	18%
4	*Enterobacter species*	4	8%
5	*staphylococcus species*	2	4%

Staphylococcus aureus reports the lowest frequency of isolation 2 (4%) of the *citrobacter species* 6 were identified as *Citrobacter koseri* and 3 were identified as *Citrobacter freundii* results showed that a high percentage of organisms were isolated from both males and females within the age bracket 0-25, 26-50 and 51-75 years. However there were more cases in females than males. (Table 3).

TABLE 3: PREVALENCE OF UTI INFECTION BY AGE & SEX

AGE	SEX			
	MALE		FEMAL	
	NUMBER OF INFECTION	TOTAL PERCENTAGE	NUMBER OF INFECTION	TOTAL PERCENTAGE
0-25	6	12	8	16
26-50	4	8	15	30
51-75	3	6	11	22
76-100	1	2	2	4

Among 50 urine samples, Isolates were identified by their cultural morphological and Bio-chemical characters (Table 4).

TABLE: 4 MORPHOLOGICAL AND CULTURAL CHARACTERISTICS OF ORGANISMS

S. NO	ORGANISMS	GRAM STAINING	HANGING DROP METHOD	NUTRIENT AGAR	MAC CONKEY AGAR
1	*Escherichia coli*	-	+	Convex, Tiny	Pink, Tiny LF
2	*Klebsiella SPP*	-	-	Mucoid, Concave	Pink, Mucoid Large colonies
3	*Citrobacter koseri*	-	+	small, convex	Pink very Tiny
4	*Citrobacter freundii*	-	+	Small, Circular	Convex, dark coluies Tiny NLF
5	*Enterobacter spp*	-	+	Wet, grey colour colonies	Tiny LF, Pink
6	*Staphylococcus aureus*	-	+	Small convex	colour colnies

The isotes was confired based on the bio-chemical tests such as Indole production, Methyl-red test, voges proskauer test, citrate utilization test urease test, Mannitol fermentation test, H_2S production and Triple – sugar ion test (Table 5).

Of thea citrobacter isolates 6 were identified as *Citrobacter koseri* and 3 were identified as *Citrobacter freundii.* (Table 6).

TABLE: 6 OCCURRENCE OF CITROBACTER SPP IN THE URINE ISOLATES

S.NO	CITROBACTER SPP	NO.OF ISOLATES	PERCENTAGE
1	*Citrobacter Koseri*	6	66.6%
2	*Citrobacter freundii*	3	33.3%

5 BIO-CHEMICAL CHARACTERIZATION OF ISOLATES

S.NO	TEST	E.coli	Klebsiella Pneumoniae	Citrobacter Koseri	Citrobacter freundii	Eterobacter spp	Staphylococcus aureus
1	Indole	+	-	+	-	-	+
2	MR	+	V	+	+	+	+
3	VP	+	+	+	-	+	-
4	Citrate	+	V	+	+	+	+
5	Urease	+	V	+	+	+	+
6	Mannitol Fermentation	+	+	+	+	+	+
7	H_2S Production	-	-	-	+	-	-
8	TSI	A/A G^+	A/A G^+	A/A G^+	A/A G^+	A/A G^+	A/A G^+

TABLE: 7 PERCENTAGE OF ANTIBIOTIC SENSITIVITY PATTERNS OF ISOLATED ORGANISMS

ORGANISM	No. Of Tested	Amitoin 30mcg	Amexyclav 30mcg	Ceftazictime 30 mcg	Cefotaxime 30mcg	Ampicillin 10 mcg	Tobromycin 10 mcg	Ciprofloxacin 5 mcg	cefuroxime 30 mcg	Doxycyctre	Gentamycin 10mcg	Kanomycin 30 mcg	Roxythromycin 30mcg	Levofloxacin 5 mcg	cefaclor 30 mcg	cephalexin 30 mcg	Gatifloxcin 5 mcg	Nitrofurantoin 10mcg
E.coli	25	68	20	20	32	12	40	4	8	12	28	24	16	38	8	24	4	84
Klebsiella spp	10	50	0	10	10	0	50	20	20	20	40	20	20	20	0	40	0	50
Citrobacter spp	9	77.7	0	22.2	44.4	0	33.3	0	0	11.1	44.4	66.6	11.1	22.2	0	11.1	11.1	77.7
Enterobacter spp	4	75	0	50	25	0	50	0	0	0	0	50	50	75	0	0	0	75
staphylococcus spp	2	50	0	100	100	0	50	0	50	50	5	50	0	0	50	50	50	50

TABLE: 8 PERCENTAGE OF ANTIBIOTIC RESISTANT PATTERNS OF ISOLATED ORGANISMS

ORGANISM	No. Of Tested	Amitoin 30mcg	Amexyclav 30mcg	Ceftazictime 30 mcg	Cefotaxime 30mcg	Ampicillin 10 mcg	Tobromycin 10 mcg	Ciprofloxacin 5 mcg	cefuroxime 30 mcg	Doxycyctre	Gentamycin 10mcg	Kanomycin 30 mcg	Roxythromycin 30mcg	Levofloxacin 5 mcg	cefaclor 30 mcg	cephalexin 30 mcg	Gatifloxcin 5 mcg	Nitrofurantoin 10mcg
E.coli	25	4	56	36	40	84	40	0	72	68	44	32	68	52	84	44	76	0
Klebsiella spp	10	0	60	60	60	90	40	5	70	50	20	30	60	40	90	40	60	30
Citrobacter spp	9	22.2	77.7	66.6	0	88.8	55.5	99.9	77.7	44.4	0	11.1	44.4	66.6	77.7	55.5	77.7	11.1
Enterobacter spp	4 2	0	100	0	0	100	25	100	100	75	50	50	50	25	10	50	75	25
staphylococcus spp		50	100	0	0	0	0	0	0	0	0	50	50	50	0	50	50	0

To detect the sensitivity and resistant percentage of the isolated organisms were done by Kirby-Bauer disc diffusion method. According to sensitivity pattern, (Table 7) Gram negative isolates suc as E.coli were mostly sensitive to Nitrofurantion 21(84%) Amikacin 17 (68%) Tobramycin 10(40%), Gentamycin 7 (28%) cefotaxime 8 (32%) levofloxacin 8 (32%), Kanamycin 6 (24%). Kelbsiella species mostly sensitive to Amikacin 5 (50%), Tobramycin 5(50%), Gentamycin 4 (40%), Nitrofurantion 5 (50%), and cefotaxime 5 (50%). Citrobacter species were mostly sensitive to Amiikacin 7 (77%) Kanamycin 6 (66%), cefotaxime 4 (44.4%) and Gentamycin 4 (44.4%). Enterobacter species were mostly sensitive to Amikacin 3 (75%), Cefazidime 2 (50%), Levofloxacin 3 (75%) and Nitrofurantoin 3 (75%).

Staphylococcus species were mostly sensitive to caftazidime 2 (100%), cefotaxime 2 (100%), Amitocin 1 (50%), Tobramycin 1 (50%), cefuroxime 1 (50%), Doxyancline 1 (50%), Cefaclor 1 (50%), cephalexin 1 (50%), nitrofurantoin 1 (50%). According to Resistant pattern, (Table 8), *E.coli* is mostly resistant to Ampicillin 21 (84%), cefaclor 21 (84%), Gatifloxacin 19 (76%), cefuroxime 18 (72%), Doxycycline 17 (68%), Amoxyclar 14 (56%) and levofloxacin 13 (52%).. *Klebsiella spp* were mostly resistant to Amplicillin 9(90%), cefaclor 9 (90%), cefuroxime 7 (70%), Amoxyclav 6 (60%), ceftazidime and cefotaxime 6 (60%), ciprofloxacin and Doxycycline 5 (50%).. *Citrobacter spp* were mostly resistant to ciprofloxacin 9 (100%), Ampicillin 8 (88.8%), Amoxyclav, cefuroxime, cefaclor and Gatifloxacin 7 (77.7%). *Enterobacter spp* were mostly resistant to amoxy-calv, ampicillin, ciprofloxacin, cefuroxime and cefaclor 4 (100%), Doxycycline and Gatifloxacin 3 (75%).

In this present study, 5 organisms were isolated among this *E.coli* was the predominant organism in UTI samples, *Klebsiella* found next to *E.coli* (Jone R.1997) E.coli exhibited lowest resistant pattern to almost of antibiotics tested. *Enterobacter* exhibited highest resistant. Based on the results, Nitrofurantoin shaved an effective antibiotic for UTI's. UTI's are more frequent in females than males. Urinary tract infections was caused primarily by gram-negative organisms than gram – positive organisms *E.coli* and *Klebsiella spp* were mostly susceptible to Nitrofurantoin and Amikacin. Amikacin and Kanamycin shows on effective antibiotic for UTI's causing *Citrobacter species.* Amikacin, Nitrofurantoin and Levofloxacin shows effective antibiotic for UTI's causing *Enterobacter spp*. Ceftazictime shows an effective drug of choice for *Staphylococcus species* causing drug of choice for *Staphylococcus species* causing urinary tract infections. This study shows that the effective antimicrobial susceptibility pattern for various organisms causing urinary tract infections.

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