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A Study of ESBL Uropathogens in A Tertiary Care Hospital with Reference to their Antibiogram

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Abstract
Background: *To Process the urine specimens received to the laboratory. The aim of the study is to determine the type of uropathogens in the region and also to study their antibiotic sensitivity in a tertiary care hospital.*
Methods *: A total of 7023 urine specimens of all age groups were processed in the central clinical microbiology laboratory of Narayana medical college for a period of 14 months from Sep 2012 to Oct 2013.Samples included of various type like Clean catch mid stream [CCMS], Cather collected, Suprapubic aspiration and nephrostomy. The specimens were inoculated on Nutrient agar, Blood agar and Macconkey agar and processed. Isolates identified by standard Biochemical tests and Antibiotic sensitivity was done by Kirby-Bauer disc diffusion method. ESBL isolates were identified by phenotypic disc diffusion method and antibiotic sensitivity was performed and results interpreted as per CLSI guidelines.*
Results: *5538 were culture positive and females were predominant over males with 3071 number as with other studies. Maximum age group in the study was between 51-60 years. Urinary tract infection was the major clinical condition followed by renal calculus, Diabetes. Escherichia coli was the predominant uropathogen in the study , Klebsiella pneumoniae, Acinetobacter sp, Pseudomonas aeruginosa, Citrobacter sp, Proteus Sp formed the rest .Enterococci sp was predominat gram positive uropathogens and rest include CONS, Staphylococcus aureus. Candida was also isolated. Gram negative uropathogens exhibited maximum sensitivity to Imipenam, Piperacillin + tazobactam and Amoxycillin+ clavulanic acid and maximum resistance to Amoxycillin,Cephalexin. E.coli exhibited moderate degree of sensitivity to Nitrofurantoin. In our study Klebsiella pneumoniae was the major ESBL uropathogen followed by Pseudomonas aeruginosa, Escherichia coli. Piperacillin+Tazobactam followed by Imipenem exhibited maximium sensitivity to ESBL uropathogens. Gram positive uropathogens demonstrated maximum sensitivity to Vancomycin and Linezolid.*
Conclusion: *Monitoring and regular screening for the production of ESBL's in the laboratory itself among uropathogens helps in prompt interventional measures in controlling ,spreading the development and dissemination of resistance in the community. The carbapnems should always be kept as reserve drugs in treatment of complicated UTI,s and in UTI,s caused by multi drug resistant uropathogens.*
Keywords: *Urinary tract infections, Uropathogens, ESBL, Multi drug resistant uropathogens.*

INTRODUCTION

Urinary tract infections [UTI's] are one of the most common infections encountered in clinical practice. As many as 35% of Nosocomial infections are UTI's explaining the significance of UTI both in community as well as hospitals[1]. However the treatment to this is dependent upon multiple factors like age, sex of the patient, underlying disease, etiological agent etc. These infections are a major concern because evidences indicate that they are responsible for major antibiotic consumption in and out of hospital[2]. Many studies have documented a clear change in the antibiotic sensitivity of uropathogens to commonly used antibiotics making empirical therapy difficult[3]. This development of resistance among uropathogens is variable from place to place, hospital to hospital making a common empirical therapy impracticable. To optimize the empirical therapy it is necessary for the clinicians to have a clear knowledge about the type of uropathogens and their antibiotic susceptibility[4]. So an area specific monitoring study to identify the Uropathogens and their susceptibility is mandatory for selecting the appropriate antibiotic therapy. The development of resistance among uropathogens is multi-factorial which may include reduced outer membrane permeability, target site modification and efflux of β-lactams out of the cell. Production of β-lactamase enzyme is the most common mechanism and extensive more common use of Third generation Cephalosporins has lead to the development of new group called as Extended spectrum Beta lactamases [ESBL].Plasmid mediated production of these group are about 300 in number and inhibited by clavulanic acid, sulbactam and tazobactam. ESBL production is more commonly observed among the gram negative uropathogens[5]. The present study was undertaken to determine the type of uropathogens prevalent in the region and also to determine their susceptibility pattern. This helps to gain the knowledge in empirical therapy of UTI. The present study also highlights the detection of ESBL strains among the isolated gram negative uropathogens.

METHODS

A prospective study was conducted over a period of 14 months from September 2012 to October 2013 in Central Microbiology Laboratory of Narayana medical college, Nellore, Andhra Pradesh. The study was approved by the institutional ethical committee. All the age groups were included in the study. The urine specimens received in the Laboratory included clean catch mid stream sample [CCMS], catheter sample, nephrostomy and suprapubic aspiration samples. All were collected as per the standard guidelines and specimens which were inadequate, improperly labeled and delayed specimens were not considered for the study. The Specimens were processed immediately by inoculating on Nutrient agar, Blood agar, Macconkey agar [Hi-media laboratories, Mumbai]. The media was incubated at 37^0c overnight and observed for significant growth [$>10^5$ CFU/ml for CCMS specimen and $>10^3$ CFU/ml for rest of the specimens].Pure growth of the organisms and individual colonies from mixed culture in significant growth were processed as individual colonies and identified by standard biochemical tests [6] . Antibiotic sensitivity test was performed on Muller-Hinton agar by Kirby-Bauer disc diffusion Method[7,8].The antibiotics(µg)which were tested for Gram negative isolates included Amoxycillin, Cephalexin, Ceftazidime, Cefotaxime, Cefixime, Nitrofurantoin,Trimethoprim+sulphomethoxazole, Ciprofloxacin, Ofloxacin, Gentamicin, Amikacin, Cefoperazone+sulbactam, Amoxyclav, Imipenam and Pencillin, Vancomycin and linezolid were placed in addition to Gram positive organisms. The sensitivity and resistance pattern were interpreted based upon the Zone size criteria as recommended by CLSI [9].

***Criteria for selection of ESBL strains*:** Isolates which were resistant to at least one of the 3rd generation Cephalosporins Eg: Cefotaxime (30µg), Ceftazidime(30µg) and Ceftriaxone(30µg) based upon CLSI criteria were suspected ESBL producers . The suspected isolates were further confirmed as ESBL strains by performing

"*Phenotypic confirmatory Disc Diffusion Test*" as per CLSI guidelines [9].

***Phenotypic Confirmatory disc diffusion Test*:** The suspected strains are subjected to individual Ceftazidime (30µg) disc along with Ceftazidime + clavulanic acid(30/10µg) combination disc on a Muller-Hinton agar plate. An increase in Zone of inhibition diameter by ≥ 5mm of combination disc when compared to Ceftazidime alone is considered to be an ESBL producer.

The confirmed Gram negative ESBL strains were further performed Antibiotic sensitivity to Imipenem (30µg), Piperacillin+tazobactam (30/10µg) and Ceftazidime+ Clavulanic acid (30/10µg) by standard Kirby-Bauer disc diffusion method and results interpreted as per CLSI guidelines.

***Quality control*:** Escherichia coli ATCC 25922 [β-lactamase negative], Klebsiella pneumoniae ATCC700603 [ESBL producer] were used as control strains. [Hi-media laboratories, Mumbai].

RESULTS

A total of 7023 specimens were processed for the isolation and identification of uropathogens. Of these 5538 showed significant bacteriuria and the rest 1485 were either insignificant bacteriuria or sterile. Table-1 illustrates the data with regard to samples received and percentage of culture positivity. Maximum culture positivity [91.22%] was observed in December followed by January [86.35%]. Of all the total 5538 culture positive cases 2467[44.6%] were males and 3071[55.4%] were females [TABLE-2]. The age group included from 0 to 90 yrs. Out of total culture positive cases maximum 850/5538 was seen during 51-60 years [15.35%] followed by 31-40yrs[14.54%] and 21-30 years[14.45%][TABLE-3& FIGURE-1].

Out of 7023 received specimens maximum cases were diagnosed as UTI 1852/7023[26.37%], followed in order by Diabetes1432/7023[20.39%], Renal calculus1343/7023[19.12%], Urethral abnormalities 987/7023[14.05%], Pregnancy 912/7023[12.99%] and last Instrumentation 321/7023[4.57%][TABLE -4].

Gram positive organisms accounted for 386/5538 [7%], Gram negative 5105/5538 [92.25%] and 47/5538[0.8%] was Candida sp. Escherichia coli was the major pathogen 2786/5105[50.3%] followed in order by Klebsiella pneumoniae [21.7%], Pseudomonas aeruginosa [10.8%], Acinetobacter baumanii [3.8%], Proteus mirabilis [2.2%], Proteus vulgaris [1.8%] and the last Citrobacter sp[1.6%]. [FIGURE-2] Enterococci sp was the predominant Gram positive uropathogen [3.2%] followed by Staphylococcus aureus [2.1%] and Coagulase Negative Staphylococcus [1.7%]. Candida Sp formed 0.8% [47/5538] among the isolates. [TABLE-5]

Antimicrobial susceptibility of both gram positive and gram negative pathogens was performed and summarized in Table 6 and 7. The most common uropathogen E.coli exhibited maximum sensitivity to Imipenem (89%) followed by Cefoperazone+sulbactam (82%) and maximum resistance was shown to Amoxycillin(82%) followed by Cephalexin(56%). Among Cephalosporins, Ceftazidime(79%), Cefixime (78%) exhibited maximum sensitivity. E.coli exhibited low susceptibility to commonly used Nitrofurantoin (72%), Gentamicin(72%), Trimethoprim+sulphomethoxazole (71%) and Ciprofloxacin(69%). Moderate degree of susceptibility to Amoxyclav(76%), Ofloxacin (78%), Amikacin(76%) was demonstrated.

Klebsiella pneumoniae demonstrated maximum ≥80% sensitivity to Imipenem, Cefoperazone +sulbactam, Ofloxacin and Ceftazadime, 70-79% sensitivity to Cefotaxime, Cefixime, Trimethoprim+sulphomethoxazole, Ciprofloxacin, Gentamicin, Amikacin,and Amoxyclav. Amoxycillin, Cephalexin, and Nitrofurantoin were least susceptible.

Non-fermenters Pseudomonas aeruginosa and Acinetobacter baumanii exhibited maximum sensitivity > 80% to Imipenem, Cefoperazone +sulbactam, Amoxyclav, Amikacin, Ofloxacin and Ceftazidime.

Other gram negative pathogens Proteus sp and Citrobacter sp demonstrated maximum sensitivity >80% to Imipenem, Cefoperazone+sulbactam, Amoxyclav, Ofloxacin, Amikacin,Cefixime and Ceftazidime.[FIGURE-3]

Gram positive pathogens exhibited maximum sensitivity >90% to Vancomycin and Linezolid and least <40% to Amoxycillin,and Cefalexin. Moderate degree of sensitivity > 70% to Amikacin, Ofloxacin, and Amoxyclav.[FIGURE-4]

All the gram negative isolates which were suspected ESBL producers were subjected to Phenotypic Confirmatory disc diffusion test. Out of 5105 suspected ESBL strains, 1805 (35.36%) confirmed ESBL production. Klebsiella pneumoniae was the major pathogen 40.43% followed in order by Pseudomonas aeruginosa 37.12%, Acinetobacter baumanii 36.20%, Escherichia coli 34.24%, Proteus mirabilis 23.14%, Proteus vulgaris 21.79% and least Citrobacter 19.54%.[TABLE-8]

All the ESBL uropathogens were further subjected to antimicrobial susceptibility to Imipenem, Piperacillin+tazobactam and Ceftazidime + clavulanicacid. ESBL producing E.coli and Klebsiella pneumoniae demonstrated maximum sensitivity to Piperacillin+tazobactam (92%) followed by Imipenem (89%) and Ceftazidime+clavulanic acid (81%). All the ESBL pathogens demonstrated maximum sensitivity to Piperacillin+tazobactam >90% followed by Imipenem >85% indicating Piperacillin +tazobactam and Imipenem as a good choice in empirical therapy of complicated UTI caused by ESBL pathogens.[TABLE-9 & FIGURE-5]

Table-1 Number Of Urine Specimens For Culture & Sensitivity From September 2012 To October 2013

MONTH	RECEIVED SAMPLES	CULTURE +VE	%
Sep-12	524	423	80.73
Oct-12	478	356	74.48
Nov-12	509	385	75.64
Dec-12	524	478	**91.22**
Jan-13	425	367	**86.35**
Feb-13	487	378	77.62
Mar-13	467	379	81.16
Apr-13	521	367	**70.44**
May-13	534	387	72.47
Jun-13	524	399	76.15
Jul-13	498	378	75.90
Aug-13	467	387	82.87
Sep-13	576	478	82.99
Oct-13	489	376	76.89
TOTAL	**7023**	**5538**	**78.86**

TABLE-2 Sex Distribution Of Culture Positives

	NO	%
MALES	2467	44.6
FEMALES	3071	55.4
TOTAL	5538	

Table-3: Age wise distribution of uropathogens isolated

Age group(yrs)	No of isolates	%
0-10	260	4.69
11-20	337	6.09
21-30	800	14.45
31-40	805	14.54
41-50	678	12.24
51-60	850	**15.35**
61-70	728	13.15
71-80	570	10.29
81-90	510	9.21
TOTAL	**5538**	100.00

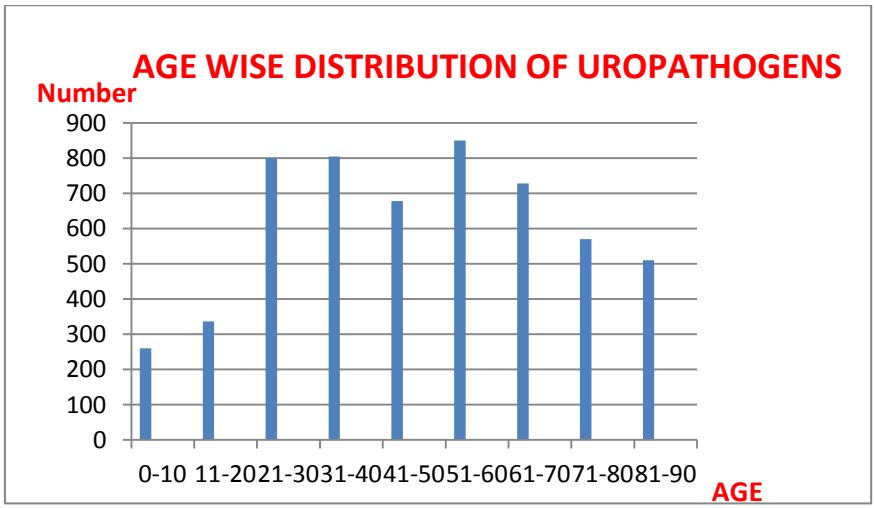
TABLE-4 Clinical information of the patients

Clinical diagnosis	No of patients	% of cases
UTI	1852	26.37
Renal calculus	1343	19.12
Urethral related abnormalities	987	14.05
Diabetes	1432	20.39
Pyelonephritis	176	2.51
Pregnancy	912	12.99
instrumentation	321	4.57
Total	**7023**	

TABLE-5: Uropathogens Isolated

Gram negative	**Number**	**%**
E.coli	2786	50.3
K.pneumoniae	1202	21.7
P.aeruginosa	598	10.8
A.baumanii	210	3.8
P.mirabilis	121	2.2
P.vulgaris	101	1.8
citrobacter	87	1.6
TOTAL	**5105**	**92.2**
Gram positive		
Enterococci	178	3.2
S.aureus	115	2.1
CONS	93	1.7
TOTAL	**386**	**7**
FUNGI		
Candida	47	0.8
TOTAL	**5538**	

Fig 1



Fig; 2

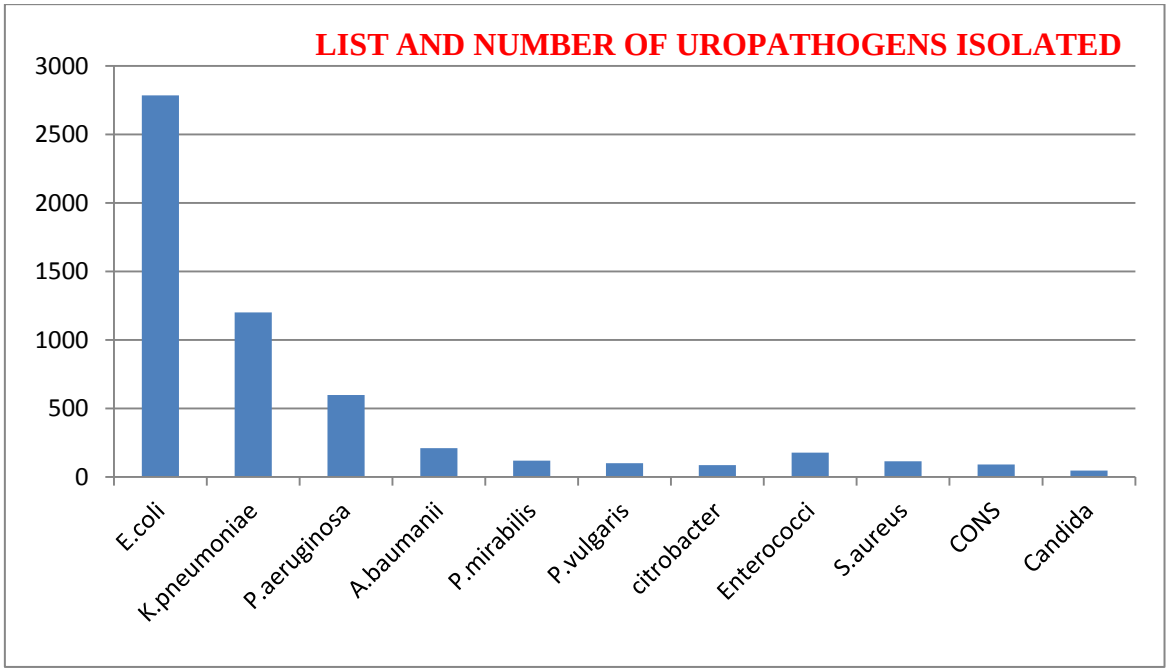


TABLE-6: Antibiogram of Gram negative isolates

ISOLATE (SENS%)	Amx	Cpxn	Ctz	Ctx	Cfx	Ntn	TSZ	Cpx	Ofx	Gtn	Akn	Cfz+sbm	Amcv	Ipm
E.coli	18	56	79	73	78	72	71	69	78	72	76	82	76	89
K.pneumoniae	21	61	80	72	79	43	72	76	82	71	75	83	79	85
P.aeruginosa	11	65	81	75	79	23	73	77	81	69	81	82	80	86
A.baumanii	17	66	82	71	80	29	78	73	81	78	81	85	81	87
P.mirabilis	16	63	81	78	81	35	79	79	82	72	80	81	80	88
P.vulgaris	19	66	82	72	82	38	72	78	80	73	80	81	80	88
citrobacter	23	73	81	81	87	45	76	79	80	81	83	86	80	89

Amx: Amoxycillin, Cpxn: Cephalexin, Ctz: Ceftazidime, Ctx: Cefotaxime, Cfx: Cefixime,Ntn: Nitrofurantoin,TSZ: Trimethoprim+sulphomethoxazole,Cpx: Ciprofloxacin,Ofx: Ofloxacin, Gtn: Gentamicin,Akn : Amikacin,Cfz+sbm: Cefoperazone+sulbactam, Amcv: Amoxyclav,Ipm: Imipenem.

Fig 3

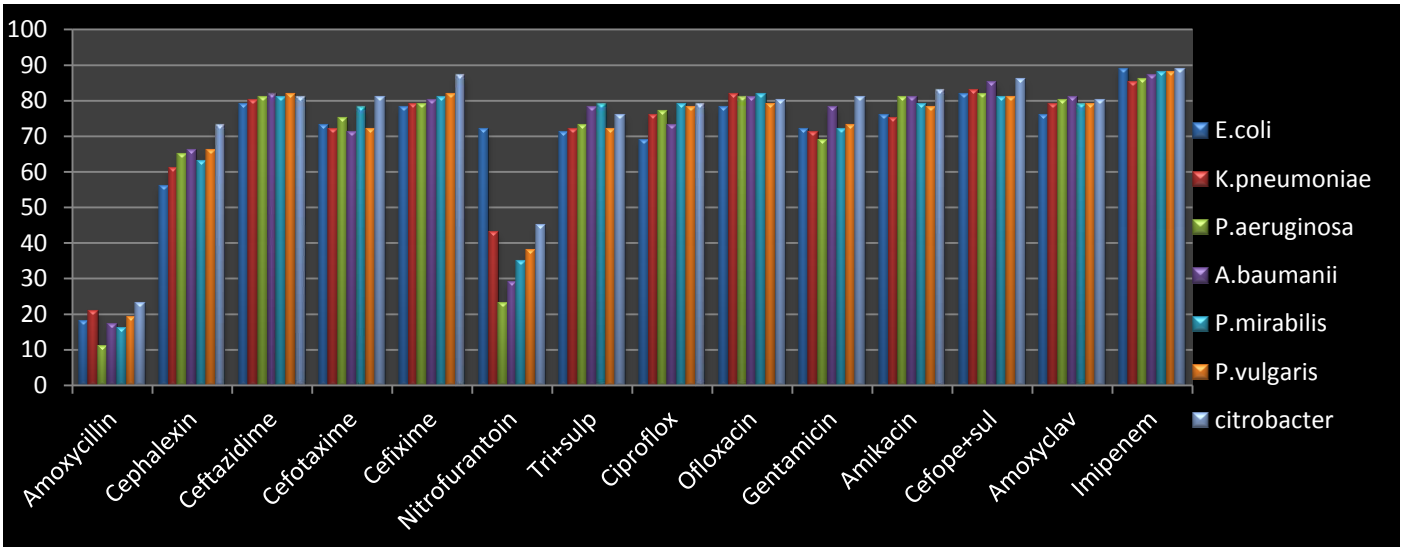


TABLE:7. Antibiogram of Gram positive organisms

ISOLATE (SENS%)	Pen	Amx	Cpx	Ofx	Gtn	Akn	Vmn	Lzd	Cfxn	Amcv	Nftn	Cfx
S.aureus	64	32	69	78	65	84	92	96	31	72	56	84
Enterococci	78	31	NT	NT	68	81	95	93	35	NT	NT	NT
CONS	63	23	71	78	64	83	93	97	37	74	51	81

Pen: Pencillin, Amx: Amoxycillin,Cpx: Ciprofloxacin,Ofx: Ofloxacin,Gtn: Gentamicin,Akn: Amikacin,Vmn: Vancomycin;Lzd: Linezolid, Cfxn: Cephalexin, Amcv: Amoxyclav,Nftn: Nitrofurantoin,Cfx:Cefixime ***NT: Not Tested.**

Fig 4

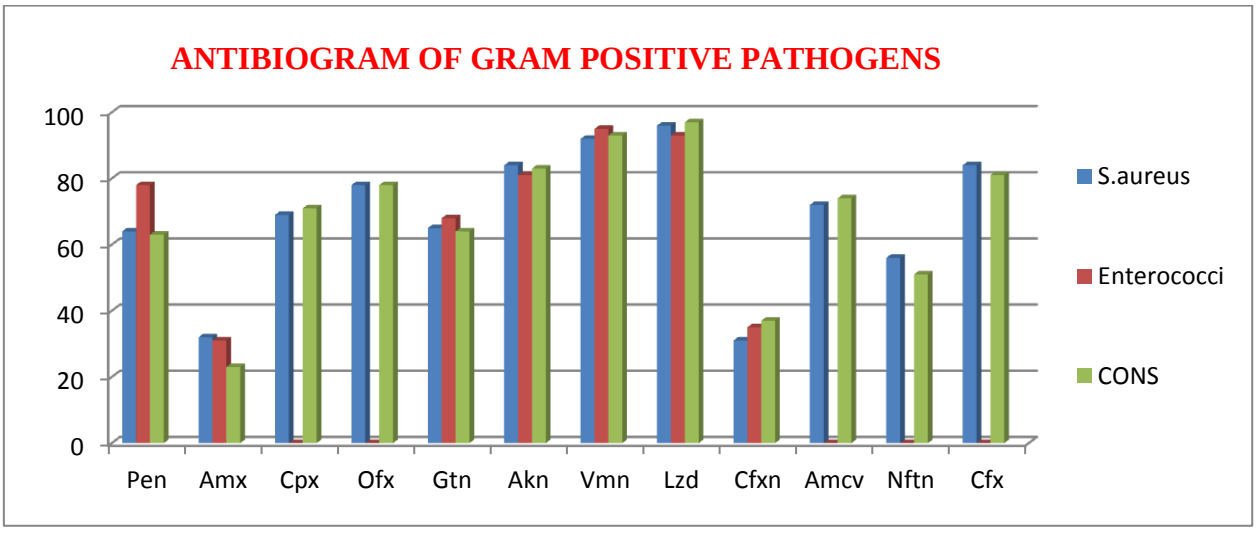


TABLE: 8 Distribution of ESBL Gram negative Uropathogens

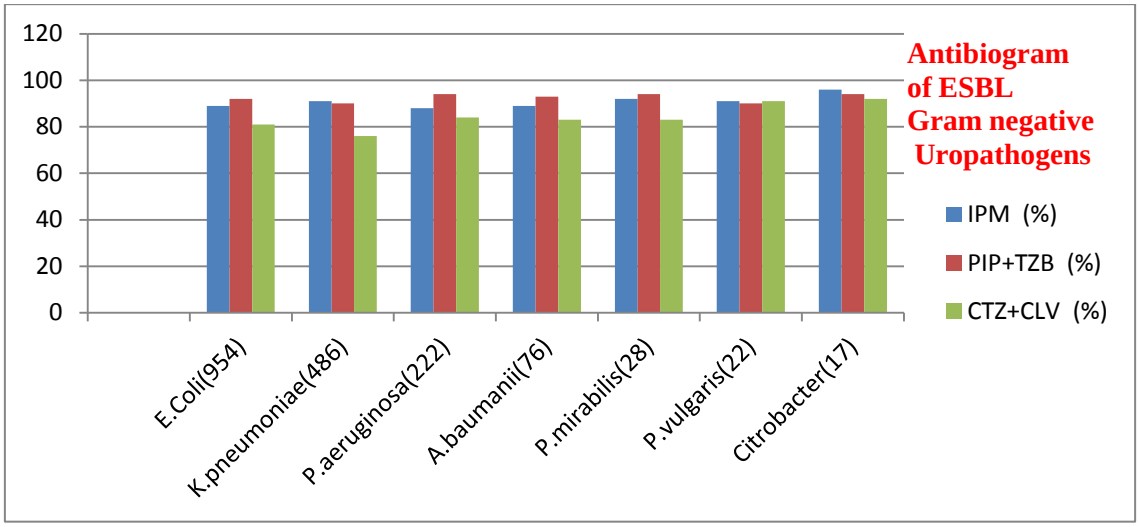
Name of Isolate(no)	Esbl strain	
	Prod(%)	**Non-Prod(%)**
E.Coli (2786)	954 (34.24)	1832 (65.76)
K.pneumoniae(1202)	486 (40.43)	716 (59.56)
P.aeruginosa(598)	222 (37.12)	376 (62.88)
A.baumanii(210)	76 (36.20)	134 (63.80)
P.mirabilis(121)	28 (23.14)	93 (76.86)
P.vulgaris(101)	22 (21.79)	79 (78.21)
Citrobacter(87)	17 (19.54)	70 (80.45)
TOTAL(5105)	**1805**	**3300**
	35.36%	**64.64%**

TABLE:9 Antibiogram of ESBL Gram negative uropathogens

Name of Isolate(NO)	IPM (%)	PIP+TZB (%)	CTZ+CLV (%)
E.Coli(954)	89	92	81
K.pneumoniae(486)	91	90	76
P.aeruginosa(222)	88	94	84
A.baumanii(76)	89	93	83
P.mirabilis(28)	92	94	83
P.vulgaris(22)	91	90	91
Citrobacter(17)	96	94	92

Ipm: Imipenem; Pip+ Tzb: Piperacillin+ tazobactam, Ctz+clv: Ceftazidime+ clavulanic acid

Fig 5



DISCUSSION

The study demonstrated the changing trends of the uropathogens with regard to their antibiotic susceptibility during the period. As mentioned in many studies throughout the world, female preponderance was seen in our study also forming around 55.4% when compared to males with 44.6%. This is clearly explained by many associated risk factors like short size of urethra, Periodic menstruation, hormonal influences and Physiological state like pregnancy [10,11]. With regard to age distribution of cases the most common age group in the study was 51-60 years followed by31-40 years and 21-30 years which coincided with the studies of Rao et al [12] and many others, however sex wise incidence was not done because of less number of female patients aged more than 60 years observed during the period. The cause of UTI in males less than 30 years is less common but after 50 years risk is increased because of prostatic enlargement and associated renal conditions like calculus etc. In the present study with regard to the clinical condition of the patient from whom uropathogens isolated , UTI both upper and lower accounted the major reason with 26.37%,followed by Diabetes 20.39%,Renal calculus 19.12%, Urethral related abnormalities 14.05%, Pregnancy12.99%, Instrumentation 4.57% and Pyelonephritis 2.51%. Studies in India and abroad indicate that UTI both community and hospital acquired accounts for one of the most common infections in clinical practice.

E.coli was the major Uropathogen (50.3%) in our study as indicated by studies worldwide [13,14]. Klebsiella pneumoniae was the 2nd most common 21.7% followed by Pseudomonas aeruginosa (10.8%). The other pathogens included Acinetobacter baumanii, Proteus mirabilis, Proteus vulgaris and Citrobacter sp. Among the gram positive pathogens, Enterococci was the most common (3.2%) followed by Staphylococcus aureus and Coagulase negative staphylococcus. Candida albicans was isolated from 47 cases (0.8%). The proportion of bacterial species isolated was similar to those described in many studies[15, 16].

Trimethoprim-Sulphomethoxazole, Nitrofurantoin and Fluoroquinolones were recommended in empirical therapy of uncomplicated UTI by studies of Warren JW et al, [17] and Karlowsky JA et al [18]. However in our study significant resistance was noted to all, Nitrofurantoin (40 - 70%) and Trimethoprim+Sulphomethoxazole (70-80%).

In the present study Ciprofloxacin and Ofloxacin were tested among fluoroquinolones and more degree of resistance was noted to Ciprofloxacin. This may be explained by wide spread irrational usage of ciprofloxacin locally for empirical

therapy. In our study 70% of E.coli strains were sensitive to Nitrofurantoin whereas other Gram negative & gram positive pathogens demonstrated significant resistance. However this drug is not recommended for serious upper urinary tract infections or with systemic involvement [19]. Gentamicin and Amikacin were studied among Aminiglycosides and good degree of sensitivity>80% was noted to Amikacin among the gram negative uropathogens which is consistent with the studies of Kothari A et al [20] and Guptha N et al [21].

Maximum sensitivity to all uropathogens was seen towards Imipenem and Cefoperazone+Sulbactam (>80%) in our study whereas moderate degree towards Amoxyclav (>75% - <80%). The trend of decreased sensitivity to Amoxyclav and emergence of resistance towards Imipenem is seen because of irrational prescription of Amoxyclav and Imipenem even in moderate UTIs. These findings are in concordance with studies of Kothari et al and Guptha N et al and many other studies abroad.

In our study 35.36% of the gram negative pathogens were ESBL producers indicating the serious concern. This percentage of ESBL is usually high when compared with the previous studies from India which reports the prevalence to be 6.6% to 68%.Subha et al reported the incidence as 6.6% in Klebsiella pneumoniae from children [22] whereas Babypadmini et al reported 40.3% in their study [23] which indicates the variability among ESBL producers from region to region. In our present study Klebsiella pneumoniae was the major ESBL producer with 40.43% followed in order by Pseudomonas aeruginosa 37.12%, Acinetobacterbaumanii 36.20%, E.coli 34.24% , Proteus mirabilis 23.14% ,Proteus vulgaris 21.79% and Citrobacter Sp 19.54% which correlated well with the studies of Mathur et al, C.Rodrigues et al and Singal et al.[24,25,26]

However few studies mentions E.coli as the major ESBL producer [27,28]. All the ESBL producers were subjected to Antibiotic sensitivity against Imipenem, Piperacillin+tazobactam and Ceftazidime+clavulanic acid. ESBL producers exhibited maximum sensitivity to Piperacillin +tazobactam (>90%) followed by Imipenem (>85%)which correlates with the studies of Tankhiwale SS et al.[29]

Inappropriate antibiotic usage, lack of proper hygiene, immunosupression and prolonged hospital stay are some of major etiological factors that enhance the chances of UTI.

CONCLUSION

Regular monitoring of the uropathogens and their antibiotic sensitivity should be done for staring empirical therapy in all cases of UTIs. Irrational misuse of antibiotics lead to development of multi drug resistance among the uropathogens. Monitoring and regular screening for the production of ESBL's in the laboratory itself among uropathogens helps in prompt interventional measures in controlling ,spreading the development and dissemination of resistance in the community. The carbapnems should always be kept as reserve drugs in treatment of complicated UTI,s and in UTI,s caused by multi drug resistant uropathogens.

CONFLICT OF INTEREST: NONE

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