Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 9, Issue, 03, pp.48329-48334, March, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

NEED OF AREA-SPECIFIC MONITORING OF ANTIMICROBIAL SENSITIVITY OF UROPATHOGENS ESPECIALLY ESBL PRODUCERS: A LESSON FROM MALDA, WEST BENGAL, INDIA

***,[1]Dr. Arindam Karmakar, [2]Dr. Asis Manna, [3]Dr. Manali Sinharay, [1]Dr. Krishnendu Das and [4]Dr. Arnab Kumar Mandal**

[1]Department of Pathology, Malda Medical College, Malda, West Bengal, India
[2]Department of Microbiology, Midnapur Medical College, Midnapur, West Bengal, India
[3]Department of Biochemistry, Calcutta National Medical College, Kolkata, West Bengal, India
[4]Department of Microbiology, Malda Medical College, Malda, West Bengal, India

ARTICLE INFO

Article History:
Received 23rd December, 2016
Received in revised form
24th January, 2017
Accepted 26th February, 2017
Published online 31st March, 2017

Key words:

Antibiotic,
ESBL producer,
Sensitivity,
Uropathogen.

ABSTRACT

Antibiotic resistance of urinary pathogens especially the extended-spectrum β-lactamase (ESBL) producing organisms has emerged as a global challenge to clinicians trying to formulate an empirical therapy. Choice of the correct antimicrobial agent and constructing an antibiotic policy has also become very difficult in recent years. Area-specific monitoring studies are essential to gather knowledge of the prevailing microbes and their sensitivity patterns. The present endeavor studies the antibiotic sensitivity pattern of 468 culture-positive urine samples amongst a total of 4466 samples from outdoor patients from Malda, a small town in West Bengal, India. Females of reproductive age group were mostly affected. Gram negative organisms dominated the organisms with Escherichia coli having the greatest share accompanied by Klebsiella sp, Proteus sp, Pseudomonas sp etc. Fluoroquinolones were only moderately sensitive (50-60% sensitivity) against Gram negative organisms. Trimethoprim-sulphamethoxazole and nitrofurantoin also had unsatisfactory levels of sensitivity (<50%). Carbapenems, aminoglycosides and tetracyclines were consistently sensitive against most organisms. Amongst the 441 organisms tested for ESBL 241 were ESBL positive. Most of the ESBL negative organisms were susceptible to ampicillin-sulbactam and piperacillin-sulbactam. Staphylococcus aureus was reasonably sensitive to co-amoxiclav, nitrofurantoin, cotrimoxazole, tetracyclines, vancomycin, linezolid and clindamycin. Fluoroquinolones which are presently being prescribed widely have lost their effectiveness. Overall, very few choices of oral antibiotics remain to be empirically prescribed for urinary infections. ESBL positive gram negative organisms are very much on the rise.

Citation: Dr. Arindam Karmakar, Dr. Asis Manna, Dr. Manali Sinharay, Dr. Krishnendu Das and Dr. Arnab Kumar Mandal, 2017. "Need of area-specific monitoring of antimicrobial sensitivity of Uropathogens especially ESBL producers: A lesson from Malda, West Bengal, India", *International Journal of Current Research*, 9, (03), 48329-48334.

INTRODUCTION

Urinary tract infection (UTI) is one of the leading causes of bacterial infections in humans both in the community and hospital setting. (Tice, 1999; Clarridge *et al*., 1998; Sussman, 1998) In almost all cases there is a need to start treatment before the final microbiological results are available. Antibiotic resistance is an ever growing problem that is a cause of major concern on a global scale. (Wise *et al*., 1998) So it is imperative that area-specific monitoring studies aimed to gain knowledge about the type of pathogens responsible for UTIs and their resistance patterns are essential for guiding the clinician in choosing the right empirical treatment. The importance of constant monitoring of the changing patterns of antimicrobial resistance by different bacteria was stressed by Manna *et al* also. (Manna *et al*., 2006) β-Lactam agents such as penicillins, cephalosporins, monobactams and carbapenems are among the most frequently prescribed antibiotic groups worldwide. β-lactamases remain the most important contributing factor to the β-lactam resistance of Gram-negative pathogens, and their increasing prevalence, as well as their alarming evolution seems to be directly linked to the clinical use of novel sub-classes of β-lactams. (Medeiros, 1997) β-Lactamases are bacterial enzymes that inactivate β-lactam antibiotics by hydrolysis, which result in ineffective compounds. (Bush, 2001) At least 400 different types of β-lactamases, originating from clinical isolates, have been described. (Jacoby and Munoz-Price, 2005) Since their advent in the 1980s the third generation cephalosporins were successful against most of the β-lactamase producing

Corresponding author:* **Dr. Arindam Karmakar,
Department of Pathology, Malda Medical College, Malda, West Bengal, India.

organisms and in addition had a better side-effect profile. (Paterson and Bonomo, 2005) But soon their period of victory was cut short by emergence of organisms producing extended spectrum β-lactamases which are perhaps the most severe problem for the clinicians in making the choice of antibiotics in UTIs especially in the community settings. There is no universally accepted definition of ESBLs. A commonly used working definition is that the ESBLs are β-lactamases capable of conferring bacterial resistance to the penicillins, first-, second-, and third-generation cephalosporins, and aztreonam (but not the cephamycins or carbapenems) by hydrolysis of these antibiotics, and which are inhibited by β-lactamase inhibitors such as clavulanic acid. In this article, the term ESBL will be taken to mean those β -lactamases of Bush-Jacoby-Medeiros group 2be and those of group 2d which share most of the fundamental properties of group 2be enzymes. (Bush *et al*., 1995) The aim of this study was to assess the epidemiological profile of urinary pathogens in samples arriving from in and around Malda, West Bengal in an NABL (National Accreditation Board for Testing and Calibration Laboratories)-accredited diagnostic laboratory during one year time period. It also describes the antibiotic susceptibility pattern of urinary pathogens with special emphasis on the ESBL producing organisms.

MATERIALS AND METHODS

Sample collection and analysis

The study was conducted on patients coming from in and around Malda, West Bengal attending on outpatient basis to a diagnostic laboratory during one year time period (September 2012 to August 2013). All persons undergoing urine culture procedure (total 4466) irrespective of age, sex, race etc were included in the study. Following standard procedures, freshly voided midstream clean catch specimens of urine were collected in sterile containers provided by the laboratory. (Collee *et al*., 2012) Semi-quantitative urine culture using a calibrated loop was used to inoculate Blood agar and MacConkey plates. (Collee *et al*., 2012) Following the recommendations of Kass in distinguishing genuine UTI from contamination, significant monomicrobial bacteriuria was defined as culture of a single bacterial species at a concentration of $>10^5$ cfu/ml. (Kass, 1957; Leigh and Williams, 1964) Only a single positive culture per patient was included in the analysis. The significant pathogens were identified by Gram stain followed by VITEK® 2 Compact System identification kits (Gram negative -GN ID Card, Product number 21341 and Gram positive -GP ID Card, Product number 21342). Antibiotic susceptibility testing was performed using VITEK® 2 Compact System AST cards (ASTP628 for Gram positive organisms; ASTN280 for lactose fermenting Gram negative organisms; ASTN281 for non-lactose fermenting Gram negative organisms. Bacterial identification and antibiotic susceptibility quality control were performed using the following strains:

- *Escherichia coli* ATCC 25922 (beta-lactamase negative)
- *Staphylococcus aureus* ATCC 25923 (beta-lactamase negative, oxacillin susceptible)
- *Pseudomonas aeruginosa* ATCC 27853 (for aminoglycosides)
- *Enterococcus faecalis* ATCC 29212 (for checking of thymidine or thymine level of MHA)

For detection of ESBL producing strains of *Klebsiella pneumoniae, Klebsiella oxytoca, Escherichia coli,* and *Proteus mirabilis,* Ceftazidime and Cefotaxime for screening purpose and screen positive organisms were subjected to confirmatory test using Cefotaxime-clavulanic acid and Ceftazidime-clavulanic acid. To resolve ambiguities of result in broth dilution method and as suggested by clinicians, some antibiotic sensitivity tests were done additionally by Kirby-Bauer disc diffusion method by following standard protocols of CLSI2013.

RESULTS

Epidemiology: Of the 4466 urine samples processed 468 (10.48%) gave significant growth of pathogens. Patients of all age group were included in the study. More organisms were isolated from women (61.97 %) than from men (38.03 %). More cases of UTIs were recorded among young and middle age patients (18-60 years, 64.3%). Pediatric patients (new born to 18 years) comprised 13.0% and elderly (above 60 years) constituted 22.7% of the total number.

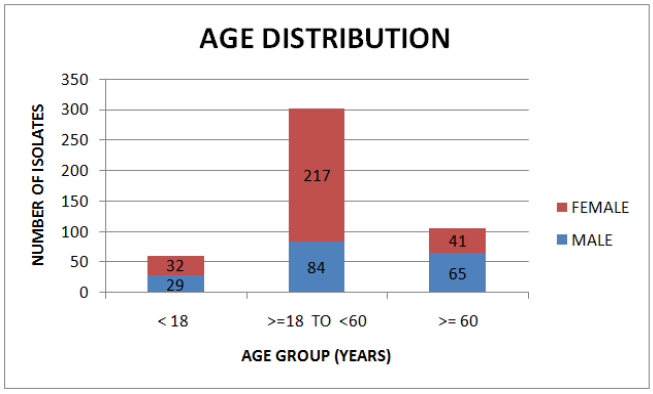

Chart 1

Of the 468 significant isolates, Gram-negative aerobic rods accounted for 458 (97.8%) while Gram-positive cocci accounted for only 10 (2.2%) of the total pathogens. The frequency and distribution of the different microorganisms are summarized in Table 1. *E. coli* (81.4%), *K. pneumoniae* (6.2%), *P. aeruginosa* (3.0%), *E. cloacae* complex (2.6%), *P. mirabilis* (1.3%), *S. aureus* (1.1%) and *Citrobacter* spp. (0.6%) were the most prevalent microorganisms in UTI patients. As depicted in Table 1, age and gender wise data of prevalence of uropathogens show that *Proteus mirabilis* infection was restricted to the adult population (more than 18 years). *Staphylococcus aureus, Acinetobacter baumannii, Citrobacter sp*, *Enterobacter aerogenenes Staphylococcus haemolyticus, Klebsiella oxytoca, Proteus vulgaris, Providencia rettgeri* and *Streptococcus agalactiae* infections were confined to the 18 to 60 years age group. It is also seen that Staphylococcus aureus infections were exclusively found in the adult females (18-60 years group). Moreover, *Edwardsiella spp.* and *Ps. putida in*fections were found only in pediatric patients (NB to 18 years) whereas *Enterococcuss spp.* and *Morganella spp.* infections were restricted to the elderly male population.

Antibiotic sensitivity

On scrutiny of the antibiotic sensitivity of the major organisms it was found that (Table 2) fluoroquinolones, as a group, was not adequately effective against the major organisms with a range of 20-50% of the organisms being sensitive.

Table 1. Frequency and distribution of the different microorganisms

Organism	<18		>=18, <60		>=60		TOTAL	
	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE	NO	%
Escherichia coli	23	29	68	180	44	37	381	81.4
Klebsiella pneumoniae	2	2	3	13	5	4	29	6.2
Pseudomonas aeruginosa	1	0	3	3	7	0	14	3
Enterobacter cloacae complex	1	1	3	4	3	0	12	2.6
Proteus mirabilis	0	0	1	4	1	0	6	1.3
Staphylococcus aureus	0	0	0	5	0	0	5	1.1
Acinetobacter baumannii complex	0	0	2	0	0	0	2	0.4
Citrobacter koseri	0	0	1	1	0	0	2	0.4
Enterobacter aerogenes	0	0	0	2	0	0	2	0.4
Enterococcus faecalis	0	0	0	0	2	0	2	0.4
Morganella morganii morganii	0	0	0	0	2	0	2	0.4
Serratia odorifera	0	0	0	1	1	0	2	0.4
Staphylococcus haemolyticus	0	0	0	2	0	0	2	0.4
Citrobacter braakii	0	0	1	0	0	0	1	0.2
Edwardsiella hoshinae	1	0	0	0	0	0	1	0.2
Klebsiella oxytoca	0	0	0	1	0	0	1	0.2
Proteus vulgaris gr.	0	0	1	0	0	0	1	0.2
Providencia rettgeri	0	0	1	0	0	0	1	0.2
Pseudomonas putida	1	0	0	0	0	0	1	0.2
Streptococcus agalactiae	0	0	0	1	0	0	1	0.2
TOTAL	29	32	84	217	65	41	468	100

Table 2. Antibiotic sensitivity pattern of all organisms (excluding beta lactams and related agents)

	FQ			AMG			CRB			TYS		CXZ	NFT
	CIP	LEV	MXI	AMI	GNT	TBR	IMP	MRP	ERP	TCY	TGY	CXZ	NFT
E. coli (381)	165	167	192	362	298	254	318	318	300	373	373	130	236
%	43.3	43.8	50.4	95.0	78.2	66.7	83.5	83.5	78.7	97.9	97.9	34.1	61.9
K. pneumoniae (29)	19	18	18	22	22	22	22	22	17	26	26	12	16
%	65.5	62.1	62.1	75.9	75.9	75.9	75.9	75.9	58.6	89.7	89.7	41.4	55.2
P.aeruginosa(14)	4	4	3	9	8	10	9	9	9	2	2	2	2
%	28.6	28.6	21.4	64.3	57.1	71.4	64.3	64.3	64.3	14.3	14.3	14.3	14.3
Enterobacter cloacae complex(12)	3	3	3	5	4	4	8	8	4	9	9	4	4
%	25	25	25	41.7	33.3	33.3	66.7	66.7	33.3	75.0	75.0	33.3	33.3
Proteus mirabilis(6)	4	2	2	6	5	5	6	6	6	6	6	3	2
%	66.7	33.3	33.3	100.0	83.3	83.3	100.0	100.0	100.0	100.0	100.0	50.0	33.3
S. aureus (5)	1	2	2	NR	NR	NR	2	2	NR	3	5	3	5
%	20	40	40				40	40		60	100	60	100

FQ- FLUORROQUINOLONES, CIP-CIPROFLOXACIN, LEV-LEVOFLOXACIN, MXI-MOXIFLOXACIN; AMG-AMINOGLYCOSIDES, AMI-AMIKACIN, GNT-GENTAMICIN, TBR- TOBRAMYCIN; CRB-CARBAPENEMS, IMP- IMIPENEM, MRP-MEROPENEM, ERP-ERTAPENEM; TYS-TETRACYCLINES, TCY-TETRACYCLINE, TGY-TIGECYCLINE; CXZ-COTRIMOXAZOLE; NFT-NITROFURANTOIN

Table 3. ESBL producing organisms

	No. of Isolates	ESBL positive	Percentage
ORGANISM TESTED FOR ESBL = 441			
Escherichia coli (381)	381	207	54.3
Klebsiella pneumoniae (29)	29	17	58.6
Enterobacter cloacae complex(12)	12	9	75.0
Proteus mirabilis(6)	6	1	16.7
OTHERS	13	7	53.8
TOTAL ESBL +	441	241	

Table 4. ESBL negative organisms' sensitivity to beta lactams

Total = 200	Amoxicillin	Ampicillin	Amoxicillin/clavulanic acid	Aztreonam	Cefazolin	Ceftriaxone	Cefotaxime	Cefepime	Ceftazidime	Cefotaxime-clavulanate	Ceftazidime-clavulanate	Ampicillin-sulbactam	Piperacillin	Piperacillin/tazobactam
Overall sensitivity – number	86	86	200	172	171	158	184	175	200	200	200	136	147	154
Overall sensitivity - %	43	43	100	86	86	79	92	87.5	100	100	100	68	73.5	76.5
ORGANISM (200)														
Escherichia coli (174)	73	73	174	149	147	135	159	154	174	174	174	114	125	130
%	42.0	42.0	100.0	85.6	84.5	77.6	91.4	88.5	100.0	100.0	100.0	65.5	71.8	74.7
Klebsiella pneumoniae (12)	0	0	12	9	11	10	11	9	12	12	12	9	9	10
%	0.0	0.0	100.0	75.0	91.7	83.3	91.7	75.0	100.0	100.0	100.0	75.0	75.0	83.3
Enterobacter cloacae complex(3)	3	3	3	3	3	3	3	3	3	3	3	3	3	3
%	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Proteus mirabilis(5)	5	5	5	5	5	5	5	5	5	5	5	5	5	5
%	100	100	100	100	100	100	100	100	100	100	100	100	100	100

Table 5. ESBL positive organisms- sensitivity to antibiotics other than beta lactams

	Fluoroquinolones			Aminoglycosides			Carbapenems			Tetracyclines		Trimethoprim/sulfamethoxazole	Nitrofurantoin		
	Ciprofloxacin	Levofloxacin	Moxifloxacin	Amikacin	Gentamicin	Tobramycin	Imipenem	Meropenem	Ertapenem	Te-tetracycline	Tigecycline	Trimethoprim/sulfamethoxazole	Nitrofurantoin	Piperacillin	Piperacillin/tazobactam
ORGANISM (241)	61	62	87	216	160	120	172	171	157	228	228	54	106	1	65
%	25.3	25.7	36.1	89.6	66.4	49.8	71.4	71.0	65.1	94.6	94.6	22.4	44.0	0.4	27.0

Table 6. Antibiotic sensitivity pattern of Gram positive organisms

	Ampicillin	Amoxicillin/clavulanic acid	Amoxicillin	Oxacillin	Benzyl penicillin	Cefoxitin	Cefalotin	Cefazolin	Cefuroxime	Piperacillin	Piperacillin+tazobactam	Azitromycin	Erythromycin	Ciprofloxacin	Levofloxacin	Moxifloxacin	Nitrofurantoin	Imipenem	Meropenem	Trimethoprim/sulfamethoxazole	Tetracycline	Tigecycline	Vancomycin	Linezolid	Quinupristin/dalfopristin	Rifampicin	Cm-clindamycin	Hlg-gentamicin high level (synergy)	Hls-streptomycin high level (synergy)
Staph aureus (5)	0	4	0	2	1	2	2	2	2	0	2	1	1	2	2	2	5	2	2	3	3	5	5	4	5	5	3	NR	NR
Staph haemolyticus (2)	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	1	1	2	2	2	2	2		
Strepto agalactiae (1)	1	1	1	1	1	NR	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	NR	NR	NR	NR	NR
Entero. Faecalis (2)	2	2	2	2	0	NR	NR	NR	NR	2	2	1	1	1	1	1	2	1	1	NR	1	2	2	2		NR	NR	1	1

Moxifloxacin was the most effective agent with around 50-60% sensitivity towards *E. coli* and *K. pneumoniae*. Aminoglycosides were reasonably effective against *E. coli*, *K. pneumoniae*, *Proteus mirabilis* (above 75%) with amikacin showing the best performance. *Pseudomonas sp.* showed somewhat less sensitivity (around 60-65%) and *E. cloacae* complex organisms were mostly resistant (with only 30-40% sensitivity). Carbapenems were consistently effective against *E. coli, K. pneumoniae, Proteus mirabilis* (sensitivity of 75-100%) with imipenem being the best performer. Pseudomonas sp. and *E. cloacae* complex organisms were little less sensitive to them (65-70% sensitivity). Tetracylines were consistent performers against *E. coli*, *K. pneumoniae* (90-98% sensitivity) and remarkably effective against *E. cloacae* complex organisms (65-70% sensitivity). But *Proteus mirabilis* (35-50% sensitivity) and *Pseudomonas aeruginosa* (14% sensitivity) were less sensitive to these agents. Trimethoprim-Sulfamethoxazole combination was only modestly effective against *E. coli*, *K. pneumoniae*, *P. mirabilis* and *E. cloacae* complex with 30-50% sensitivity, and the sensitivity even lower for *P. aeruginosa* (14%). Nitrofurantoin was also a modest performer only by showing 50-60% sensitivity towards *E. coli* and *K. pneumoniae* and 15-30% sensitivity towards *P. aeruginosa*, *E. cloacae* and *P. mirabilis*.

ESBL: Out of the 441 organisms tested for Extended Spectrum Beta Lactamase, 241 were positive (54.6%). *E. coli* and *K. pneumoniae* showed 54.3% and 58.6% positivity respectively whereas *E. cloacae* complex showed 75% positivity. Only 16.7% of the *P. mirabilis* showed ESBL positivity (Table 3). Amongst the ESBL negative organisms (table 4), 40-45% showed sensitivity towards ampicillin and amoxicillin whereas all of them were sensitive to co-amoxiclav. Aztreonam, cefazolin, ceftriaxone and cefepime showed 80-85% sensitivity. Cefotaxime showed about 92% sensitivity. All the ESBL negative organisms were sensitive to ceftazidime, cefotaxime-clavulanic acid and ceftazidime-clavulanic acid. Ampicilin-sulbactam showed about 65% sensitivity whereas piperacilin and piperacillin-tazobactam both showed about 75% sensitivity. *S. aureus* were mostly resistant to ampicillin, amoxicillin and benzyl penicillin (0-40% sensitivity) and also to cephalosporins, piperacillin, piperacillin-tazobactam. Macrolides, fluoroquinolones and carbapenems were not consistently effective (20-40% sensitivity). But co-amoxiclav, nitrofurantoin, cotrimoxazole, tetracyclines, vancomycin, linezolid, quinupristin/dalfopristin, rifampicin, and clindamycin showed reasonable sensitivity (Table 6).

DISCUSSION

Like other studies done in India and abroad, the present study of uropathogens shows that adult persons in the age group 18-60 years were affected more than the persons at the extremes of age. Females suffered more urinary infection than males, due to anatomic and physical factors mostly. (Akram *et al*., 2007; Amin *et al*., 2009; Kothari and Sagar, 2008) In consistence with other studies undertaken in India, more than 90% organisms were Gram negative rods (97.8%). *E coli* (81%) was the most frequently isolated organism followed by *K pneumoniae* (29%), *Pseudomonas aeruginosa* (14%), *Enterobacter spp.* (12%), *Proteus spp* and *Staph. aureus*. (Akram *et al*., 2007; Kothari and Sagar, 2008) Antibiotic resistance is a major clinical problem in treating urinary infections all over the world. The resistance to the antimicrobials has increased over the years. Resistance rates vary from country to country. Overall, isolates from Latin American countries show the lowest susceptibility rates to all antimicrobial agents followed by isolates from Asian-Pacific and European regions. Canadian strains exhibit the best susceptibility testing results on a global basis. (Gales *et al*., 2001) In the present study Enterobacteriaceae organisms showed a very high degree of resistance towards ampicillin, fluoroquinolones and cotrimoxazole in keeping with other Indian studies (Akram *et al*., 2007; Amin *et al*., 2009; Kothari and Sagar, 2008) which is in contrary to the results in studies in USA and Europe (Vromen *et al*., 1999; Kahlmeter, 2003) where these organisms were found to be a lot more sensitive. Nitrofurantoin also showed about 60% effectiveness towards the major organisms which is far less than showed by the western studies. As suggested by the European ECO.SENS STUDY, this is probably the consequence of widespread consumption of these agents over a long period of time. (Kahlmeter, 2003) In conformity with other studies, aminoglycosides, tetracycline and carbapenems showed very good sensitivity results. Compared to previous studies done in India (around 40%), ESBL producing organisms constitute somewhat larger proportion of organisms (55-60%) but in consistence with other studies, the ESBL positive organisms were mostly sensitive to carbapenems, aminoglycosides and tetracyclines (Akram *et al*., 2007; Kothari and Sagar, 2008; Babypadmini and Appalaraju, 2004).

Conclusion

A close look at the result of our study and review of other relevant studies done around the world reveals that the uropathogens isolated have lost sensitivity to any one oral antimicrobial agent to be relevant for empirical use, at least at Malda and surrounding areas which the laboratory caters to. It also demonstrates that the ESBL producing organisms are on the rise even in a peripheral small town like Malda. So this study gives a clear message that both clinicians and patients are going to face tough times if policies for judicious use of antibiotics cannot be implemented immediately.

Acknowledgments

The technicians of Dishari Health Point Private Limited, Malda for their help with the laboratory tests.

Conflicts of interest: None

REFERENCES

Akram, M., Mohammed, S., Khan, AU. (2007 March) Etiology and antibiotic resistance patterns of community-acquired urinary tract infections in J N M C Hospital Aligarh, India, *Annals of Clinical Microbiology and Antimicrobials,* 23; 6(4):1-7.

Amin, M., Mehdinejad, M., Pourdangchi, Z. (2009 July) Study of bacteria isolated from urinary tract infections and determination of their susceptibility to antibiotics, *Jundishapur Journal of Microbiology*, 2(3, Summer):118-123.

Babypadmini, S. and Appalaraju, B. 2004. Extended spectrum β-lactamases in urinary isolates of Escherichia coli and Klebsiella pneumoniae - prevalence and susceptibility pattern in a tertiary care hospital, *Indian Journal of Medical Microbiology,* 22(3):172-174.

Bush, K. 2001. New β-lactamases in gram-negative bacteria: diversity and impact on the selection of antimicrobial therapy, Clin Infect Dis 32:1085-9.

Bush, K., Jacoby, G., Medeiros, A. 1995. A functional classification scheme for beta-lactamases and its correlation with molecular structure, Antimicrob. *Agents Chemother,* 39:1211-33.

Clarridge, J., Johnson, J., Pezzlo, M. 1998. In: Weissfeld, A. (editor) Laboratory Diagnosis of Urinary Tract Infections. Washington, DC: American Society for Microbiology.

Collee, J., Duguid, J., Fraser, A., Marmion, B., Simmons, A. Laboratory strategy in the diagnosis of infective syndromes. In Collee, J., Marmion, B., Andrew, A., Simmons, A., editors. *Mackie & McCartney Practical Medical Microbiology*. 14th ed 2012; 87.

Gales, AC., Jones, RN., Turnidge, J., Rennie, R., Ramphal, R. 2001. Characterization of Pseudomonas aeruginosa Isolates: Occurrence Rates, Antimicrobial Susceptibility Patterns, and Molecular Typing in the Global SENTRY Antimicrobial Surveillance Program, 1997–1999, *Clinical Infectious Diseases,* 32(Suppl 2):S146–55.

Jacoby, G. and Munoz-Price, L. 2005 The new β-lactamases, *N Engl J Med*., 352:380-91.

Kahlmeter, G. 2003. Prevalence and antimicrobial susceptibility of pathogens in uncomplicated cystitis Europe. The ECO. SENS study, *Int J Antimicrob agents*, 22:49-52.

Kass, EH. 1957. Bacteriuria and the Diagnosis of Infections of the Urinary Tract With Observations on the Use of Methionine as a Urinary Antiseptic, *AMA Arch Intern Med.,* 100(5):709-14.

Kothari, A. and Sagar, V. 2008. Antibiotic resistance in pathogens causing community-acquired urinary tract infections in India: a multicenter study, *J Infect Developing Countries,* 2(5): 354-8.

Leigh, D. and Williams, J. 1964. Method for the detection of significant bacteriuria in large groups of patients, *J Clin Pathol.,* 17: 498-503.

Manna, A., Chatterjee, M., Bhattacharya, S., De, J., Ray, SK. and Das, P. 2006. Antimicrobial susceptibility pattern of Staphylococcus aureus from throat-swab isolates in a super-specialty hospital. (October) *Indian Medical Journal*, 100(10): 355-58.

Medeiros, A. 1997. Evolution and dissemination of b-lactamases. *Clin Infect Dis*., 24(Suppl 1):S19–S45.

Paterson, DL. and Bonomo, RA. 2005 October. Extended-Spectrum b-Lactamases: a Clinical Update, *Clinical Microbiology Reviews,* 18(4):657-86.

Sussman, M. Urinary tract infections. In Collier L, Balows A, Sussman M, editors: 1998. Topley & Wilson's Microbiology and Microbial Infections. 9th ed. London: Arnold; 601-21.

Tice, A. 1999. Short course therapy of acute cystitis: a brief review of therapeutic strategies, *Journal of Antimicrobial Chemotherapy,* 43:85-93.

Vromen, M., van der Van, A., Knols, A., Stobberingh, E. 1999. Antimicrobial resistance patterns in urinary tract isolates from nursing homes residents. Fifteen years of data reviewed, *J Antimicrob Chemother*, 44:113-116.

Wise, R., Hart, T., Cars, O., Streulens, M., Helmuth, R. and Huovinen, P. 1998. Antimicrobial resistance is a major threat to public health, *British Medical Journal*, 317:609–10.

****