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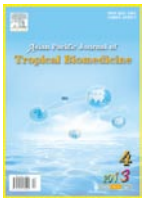

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surveillance of multidrug resistant uropathogenic bacteria in hospitalized patients in Indian

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PEER REVIEW

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Comments

This is a good study in which the authors evaluated antibiogram of notorious UTI causing bacteria isolated from clinical samples of a hospital, for a dreadful disease. Antibiograms of bacteria indicated moderately higher numbers of strains resistant to each antibiotic studied, generating the fear of precipitating fervent episodes in public health particularly with bacteria.

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ABSTRACT

Objective: To record surveillance, antibiotic resistance of uropathogens of hospitalized patients over a period of 18 months. **Methods:** Urine samples from wards and cabins were used for isolating urinary tract infection (UTI)-causing bacteria that were cultured on suitable selective media and identified by biochemical tests; and their antibiograms were ascertained by Kirby-Bauer's disc diffusion method, in each 6-month interval of the study period, using 18 antibiotics of five different classes. **Results:** From wards and cabins, 1 245 samples were collected, from which 996 strains of bacteria belonging to 11 species were isolated, during April 2011 to September 2012. Two Gram-positive, *Staphylococcus aureus* (*S. aureus*) and *Enterococcus faecalis* (*E. faecalis*), and nine Gram-negative bacteria, *Acinetobacter baumannii*, *Citrobacter* sp., *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Proteus mirabilis*, *Proteus vulgaris* and *Pseudomonas aeruginosa* were isolated. Both *S. aureus* and *E. faecalis* were vancomycin resistant, and resistant-strains of all pathogens increased in each 6-month period of study. Particularly, all Gram-negatives were resistant to nitrofurantoin and co-trimoxazole, the most preferred antibiotics of empiric therapy for UTI. **Conclusions:** Antibiograms of 11 UTI-causing bacteria recorded in this study indicated moderately higher numbers of strains resistant to each antibiotic studied, generating the fear of precipitating fervent episodes in public health particularly with bacteria, *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae* and *S. aureus*. Moreover, vancomycin resistance in strains of *S. aureus* and *E. faecalis* is a matter of concern.

KEYWORDS

UTI, MDR bacteria, Hospitalized patients, Antibiograms, Nosocomial infections, Antibiotics, *Staphylococcus aureus*, *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*

1. Introduction

According to an estimation, about 150 million reports of urinary tract infections (UTIs) per annum were recorded worldwide and about 35% of those were of nosocomial origin[1]. In the limit of course, the UTI problem has been magnified over the time with the emergence of multidrug resistant (MDR) bacteria and it has become a frequently met with medical problem. Paradigmatically, the transformation of the commensal, *Escherichia coli* (*E. coli*) mostly isolated from patients with uncomplicated UTI[2], to be a notorious pathogen is of utmost consternation. Further, its strains gained the capability of the production of extended spectrum beta-lactamase (*ESBL*) enzyme, capable of degrading antibiotics of beta-lactam and cephalosporin groups; eventually, *E. coli* strains pose an abysmal clinical annoyance, associated with development of comorbidities, high costs of hospitalization and high mortality rates[1,3], to put in sotto voce. Further, several other Gram-negative notorious UTI-bacteria are mainly

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Acinetobacter baumannii (*A. baumannii*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Proteus* sp., *Klebsiella* sp., *Chlamydia trachomatis* and *Neisseria gonorrhea*. Moreover, UTI-fungi, *Candida* sp. (such as *Candida albicans*, *Candida utilis*, *Candida glabrata*, *Candida tropicalis*, *Candida kefyr* and *Candida guilliermondii*) and *Rhodotorula* sp., often burgeon in the mazed environment of infection-source in a hospital, promoting UTI[4]. Even certain problem-causing species of *Mycoplasma* are isolated from urine samples. Indeed, a spectrum of comorbidities due to cystitis, prostatitis, pyelonephritis, urethritis and a few more are found associated with acute cases of UTI[5]. Most genital-associated infections are caused by the retrograde ascent of bacteria from fecal flora via urethra to bladder and kidney, in females[6].

A study from Tamil Nadu, India recorded predominance of bacteria as follows: *Escherichia coli* (*E. coli*) (31.5%), *Staphylococcus aureus* (*S. aureus*) (20.5%), *Klebsiella pneumoniae* (*K. pneumoniae*) (15.8%), *Pseudomonas aeruginosa* (*P. aeruginosa*) (7.5%) and *Proteus* sp. (7.4%); their strains were resistant to antibiotics (μg/disc) at decreasing levels: trimethoprim-sulphamethaxazole-30 (83.3%), nalidixic acid-30 (67.3%), amoxicillin-10 (67.3%), co-trimoxazole-10 (61%), gentamicin-10 (48.8%), ciprofloxacin-10 (46%) and cefotaxime-10 (43%), *in vitro*[7]. Empiric treatment of UTI in otherwise healthy non-pregnant females involves the use of a 3-day course of trimethoprim-sulfamethoxazole-30 μg in the US[8]. The alternate therapy for uncomplicated UTI includes nitrofurantoin or phosphomycin[9]. There were many *in vitro* studies of antimicrobial susceptibility of urinary isolates of *E. coli*[3,10]. However, the emergence of MDR strains resistant to newer and potent antimicrobials has become commonplace making therapeutic options limiting to antimicrobials, carbapenem, colistin and phosphomycin. An updated knowledge on antimicrobial susceptibility of MDR UTI path ogens is of prime importance for thwart of pugnacious issues of public health. This study elucidated that Enterobacteriaceae are the predominant UTI causing pathogens, followed by Gram-positive cocci. These findings are consistent with the earlier studies from India and Canada[11,12].

It has been recorded that one in every five women developed UTI and 95% of UTI causing organisms developed infectious complications at the notch of urethra. In sexually active women, recurrent UTIs are reported to be more frequent. Young, adolescent girls also develop cystitis or bladder infection by UTI-causing organisms[13]. UTIs are also common in patients with diabetes mellitus (DM) because this condition alters the urinogenital system, letting space/niche for the survival of a myriad of pathogens. Indeed, the most common complication is dysuria (burning sensation during urination). More often than not, the damage of the infected organ due to complicated UTI, *a priory*, with MDR strains, leads to pyelonephritis (bacterial infections up to pelvis of the kidney causing its scarring), followed by death. In case of recurrent UTI, glucosuria and impaired granulocytes formation are the associated comorbidities[14]. Most often, diabetic patients have a greater risk of the development of UTI-triggered acute pyelonephritis, renal abscess, abnormality of bladder scaring and pylities; mostly dysfunctional bladder contraction occurs during the evacuation of urine; and hospitalization for pyelonephritis is known as 15 times more frequent in UTI-cases with DM[15]. In fact, no confirmatory remedy for treatment of acute cystitis and pyelonephritis is available for patients with DM. Thus, it would be iniquitous according to principles of "comparative effectiveness research", if UTI cases are not given due attention by apothecary, with the present-day avalanche of MDR avatars of pathogenic bacteria.

This institute (IMS and Sum Hospital), has reported recently, a surveillance study of the most notorious UTI-causing bacterium, *P. aeruginosa*[16]. In face of accumulation of a vast majority of literature on MDR bacteria, it has become a matter of compulsion to conduct a regional surveillance on this exasperating class of pathogens, causing morbidity and mortality in females mainly[17]. This study recorded a gamut of antibiograms using 18 antibiotics of five groups of the time, with two Gram-positive and nine Gram-negative urinary tract associated bacteria isolated from a typical Indian hospital, a systematic study never reported before. This study should strengthen the epidemiological database of this vast subtropical country in Asia-Pacific region. It is anticipated that this work would also benefit the pharmacy-world for further strategies in the crusade of the control of MDR bacteria, as complicated UTIs prove as causes of terminal illness, leading to blood stream infections (BSI) and renal failure, mainly. The associated shenanigans of BSI are too vast to be considered herein.

2. Materials and methods

2.1. Isolation and identification of pathogenic bacteria

From hospitalized patients of wards and cabins of IMS and Sum Hospital, a total of 1 245 urine samples yielded 996 strains of pathogenic bacteria belonging to 11 species (two Gram-positive and nine Gram-negative bacteria) during the span of 18 months (from April 2011 to September 2012). All strains (*S. aureus*, *Enterococcus faecalis* (*E. faecalis*), *A. baumannii*, *Citrobacter* sp., *E. coli*, *Enterobacter aerogenes* (*E. aerogenes*), *K. pneumoniae*, *Klebsiella oxytoca* (*K. oxytoca*), *Proteus mirabilis* (*P. mirabilis*), *Proteus vulgaris* (*P. vulgaris*) and *P. aeruginosa* were identified by standard biochemical tests and were maintained as axenic cultures in suitable media. Microbial Type Culture Collection (MTCC) strain of each bacterium was used as the reference control during identification.

For pure-cultures of Gram-positive cocci, catalase and coagulase tests were performed. The catalase test was done with a drop of 3% (v/v) H_2O_2 that caused effervescence indicating the presence of catalase enzyme. For the coagulase test, a lump of a test organism was emulsified with a drop of normal saline water (0.89% v/v) and a drop of human blood serum was added to the suspension; clumping of cells was observed within 10 seconds, for confirmation of the presence of bound coagulase enzyme. When a sample of Gram-positive cocci responded positively to both catalase and coagulase tests, it was confirmed as

S. aureus. Further, catalase negative, alpha-haemolytic (partial or green haemolysis of erythrocytes) colonies were subjected to bile-esculin test. The bile-esculin medium contains esculin and peptone for nutrition, and bile to inhibit growth of Gram-positive bacteria, other than Group D streptococci or enterococci. Ferric citrate was added as a colour-indicator. Organisms, which split esculin molecules and used the liberated glucose to supply energy, release esculin into the medium. The free esculin reacts with ferric citrate in the medium to form a phenol-iron complex, which turns the agar-slant from dark brown to black. An agar-slant that was more than half darkened within 48 h of incubation was bile-esculin positive, for the confirmation of *E. faecalis*; but the alternative non-darkening of the agar was taken as the negative result[18].

2.2. Tests for pure-cultures of Gram-negative bacilli

2.2.1. Oxidase test

A bacterial colony was rubbed onto a filter paper, impregnated with tetramethyl-p-phenylenediamine dihydrochloride and the dye indophenols; the zone of the filter paper turns blue/purple in the positive result, while the negative result was with no change of colour.

2.2.2. Indole test

To get an aliquot of 5 mL 48 h old grown culture (test culture), an aliquot of 0.5 mL of Kovac's reagent (p-dimethylaminobenzaldehyde, isoamyl alcohol and HCl) was added. A formation of a cherry-red or purple-red ring at the interface of the broth culture and the reagent indicated the indole production from tryptophan by the test culture.

2.2.3. Methyl red test (MR test)

An aliquot of 5 mL sterile MRVP broth (peptone 7 g, glucose 5 g, potassium phosphate 5 g, pH 6.9, was used. The test culture was inoculated and incubated for 48 h at 37 °C. To this culture, five drops of methyl red solution were added as an indicator. If the total solution turned red, the test was taken as positive for the formation of organic acids as products.

2.2.4. Voges-Proskauer test (VP test)

To an aliquot of 5 mL sterile MRVP broth, a loopful of the test culture was inoculated and the mixture was incubated for 48 h at 37 °C. To this culture tube, 10 drops of VP I reagent (5% α-napthol, in absolute alcohol) and 2-3 drops of VP II reagent (40% KOH solution) were added and the mixture was allowed to stand for 15-20 min for the reaction to complete. The positive result was the appearance of red colour of the mixture, *i.e.*, production of a neutral product, acetoin from the fermentation of glucose by the organism, and alternately yellow colour production indicated the negative result.

2.2.5. Citrate test

The test culture was inoculated onto a slant of Simon Citrate Agar that was incubated for 48 h at 37 °C. The change of colour of agar from green to blue indicated that organism used citrate as the sole source of carbon.

2.2.6. Urease test

The test organism was inoculated onto a slant of Christensen's Urea Agar (peptone, glucose, sodium chloride, mono-potassium phosphate, urea, phenol red, distilled water, and at pH 6.8). The hydrolysis of urea yielding ammonia gas increased the pH that changes the colour of the medium from off-white to pink/orange, the positive result.

2.2.7. Triple-sugar-iron test (TSI test)

Two or three drops of test broth culture were inoculated on TSI-agar slant and subsequently, a stab was made up to the butt of the slant. The tube was incubated at 37 °C for 48 h; the black colour appearance indicated the H_2S production.

2.2.8. Nitrate test

An aliquot of 5 mL of nitrite broth (peptone 5 g, beef-extract 3 g, KNO_3 1 g and distilled water 1 000 mL) was inoculated with 1 drop of 24 h old broth test culture and was incubated for 48 h at 37 °C. From the development of red colour within 30 seconds of adding a few drops of the reagent A (α-napthol 5 g in 1 000 mL of 30% acetic acid) and reagent B (sulphanilic acid 5 g in 1 000 mL acetic acid), the positive result was inferred. No colour change suggested the negative result[18]. MTCC strain of each Gram-positive or Gram-negative bacterium was used as the reference control in each biochemical test.

2.3. Antibiotic susceptibility test

All bacterial strains including the standard MTCC strains of each bacterium were subjected to antibiotic sensitivity tests by the Kirby-Bauer's method/disc diffusion method, using a 4 mm thick Mueller-Hinton (MH) agar (HiMedia, Mumbai) medium[19]. An aliquot of 0.1 mL of 0.5 McFarland equivalents, approximately from an exponentially growing culture was spread on agar for the development of lawn of a strain of a bacterium at 37 °C in a BOD incubator (Remi CIM-12S). Further, on the lawn-agar of each plate, 8 high potency antibiotic discs (HiMedia) of 18 prescribed antibiotics of five different groups were placed, individually at equal distances from one another. Plates were incubated for 18 h at 37 °C and were examined for size-measurements of zones of inhibition around each disc, following the standard antibiotic susceptibility test chart of Clinical Laboratory Standard Institute (CLSI) guidelines[16].

3. Results

From hospitalized patients of wards and cabins, a total of 1 245 urine samples yielded 996 strains of pathogens belonging to 11 species with two Gram-positive and nine Gram-negative bacteria, during the span of 18 months. In total, there were 115 strains of *E. faecalis*, 152 strains of *S. aureus*, 72 strains of *A. baumannii*, 50 strains of *Citrobacter* sp., 194 strains of *E. coli*, 72 strains of *E. aerogenes*, 108 strains of *K. pneumoniae*, 42 strains of *K. oxytoca*, 62 strains of *P. mirabilis*, 47 strains of *P. vulgaris* and 80 strains of *P.*

aeruginosa. Thus, *E. coli* was the maximally isolated UTI causing bacterium, followed by, *S. aureus, E. faecalis, K. pneumoniae, P. aeruginosa, A. baumannii, E .aerogenes, P. mirabilis, P. vulgaris*, and *K. oxytoca* (Table 1).

Gram-positive bacteria as medium to large, smooth, entire, slightly raised, creamy yellow, green/beta-haemolytic colonies on blood agar, found positive to catalase and coagulase tests were confirmed to be *S. aureus*.

Table 1

Bacteria isolated from urine samples of the in house wards patients.

Bacteria		April-September 2011	October 2011-March 2012	April-September 2012	Total
Gram-positive	*E. faecalis*	25	45	45	115
	S. aureus	55	44	53	152
	A. baumannii	25	28	21	74
	Citrobacter sp.	14	21	15	50
	E. aerogenes	21	28	23	72
Gram-negative	*E. coli*	72	54	68	194
	K. pneumoniae	38	37	33	108
	K. oxytoca	10	17	15	42
	P. mirabilis	23	24	15	62
	P. vulgaris	19	15	13	47
	P. aeruginosa	35	28	17	80
	Grand total	337	341	318	996

Total number of urine samples was 1 245; Positive samples were 996.

Further, bile-esculin producing colonies, negative to catalase and coagulase tests were taken as *E. faecalis*, which produced grayish, round, small colonies without any haemolytic zones on blood agar. Further, the Gram-negative bacterium, *A. baumannii* was identified on colony characteristics on nutrient agar (NA), MacConkey (MC) agar and cysteine-lactose-electrolyte-deficient (CLED) agar and from results obtained from adopted biochemical procedures: it grew as colourless, smooth, opaque, raised and pinpoint colonies on NA, but as colourless, smooth, opaque, raised and non-lactose-fermenting (NLF) colonies on MC agar; it was found positive to catalase, VP and citrate tests, whereas negative to oxidase, indole, MR and nitrate tests. Similarly, *Citrobacter* sp. was identified by its colony characteristics on MC agar and results obtained from the nine biochemical tests; it produced light pink-coloured late-lactose-fermenting (LLF) colonies after an 48 h of incubation on MC agar; particularly, it was found positive to catalase, MR, citrate and nitrate tests, whereas negative to oxidase, indole, VP and urease tests. On the TSI, the bacterium produced both acid and H_2S gas during growth. Again, *E. aerogenes* produced white convex with gamma-haemolytic colonies on blood agar, and lactose fermenting (LF), and mucoid colonies on MC agar. From biochemical tests, *E. aerogenes* was seen positive to catalase, citrate, VP and nitrate tests, whereas negative to oxidase, indole, MR and urease tests. On a TSI slant, it produced acid in slant and gas production in the butt. *E. coli* produced flat dry, irregular colonies on NA; LF, flat, dry, pink and irregular colonies on MC agar; purple coloured, flat, dry, irregular colonies, with metallic green colour on eosin methylene blue agar were noted by *E. coli*, but translucent blue colonies on CLED agar were evident (Figure 1).

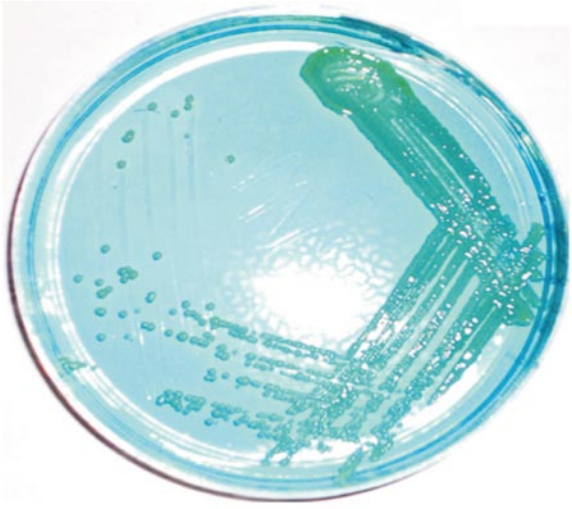

Figure 1. Translucent blue colonies of E. coli on CLED agar.

Further, *E. coli* was positive to catalase, indole, MR and nitrate tests, whereas found negative to oxidase, VP, citrate and urease tests; on the TSI test, it produced acid both in slant and butt of with gas. Both *K. pneumoniae* and *K. oxytoca* colonies on CLED agar were yellow and mucoid, whereas they produced LF, pink-coloured, mucoid colonies on MC agar. *K. pneumoniae* was found positive to catalase, VP, citrate and urease and nitrate tests, whereas it was negative to indole, MR and oxidase tests. On the TSI test, *K. pneumoniae* strains produced acids both in butt and slant along with gas production. *K. oxytoca* was found positive to the indole test, whereas the rest other test results were similar to those of *K. pneumoniae*. Similarly, both *P. mirabilis* and *P. vulgaris* had swarming and beta-haemolytic colonies on blood agar and translucent blue colonies on CLED agar. Further, *P. mirabilis* was found positive to catalase, MR, citrate and urease and nitrate tests, whereas it was negative to indole, VP, and oxidase tests. On the TSI test, *P. mirabilis* strains produced acids in both butt and slant along with H_2S gas. *P. vulgaris* was found positive to indole test, whereas the rest other results were similar to those of *P. mirabilis*. *P. aeruginosa* produced large, irregular, opaque colonies with blue-green pigment on NA; it was found positive to catalase, oxidase, urease and nitrate test, whereas negative to indole, MR and VP tests (Tables 2 and 3).

All isolated bacterial strains were subjected to antibiotic sensitivity tests with all antibiotics used, in each 6-month period. Three aminoglycoside antibiotics (μg/disc), amikacin-30, gentamicin-10 and netilmicin-30 were moderately resistant to 11 species of pathogens used, in ranges, 55% to 76% of 115 strains of *E. faecalis*, 58% to 85% of 152 strains of *S. aureus*, 47% to 64% of 74 strains of *A. baumannii*, 28% to 48% of 50 strains of *Citrobacter* sp., 52% to 81% of 72 strains of *E. aerogenes*, 51% to 81% of 194 strains of *E. coli*, 53% to 79% of 108 strains of *K. pneumoniae*, 17% to 34% of 42 strains of *K. oxytoca*, 27% to

Table 2

Media used for isolation and maintenance pathogenic bacteria from urine samples and their colony characteristics.

Bacteria	MTCC strain number	Media used	Colony characteristics
E. faecalis	439	Blood agar	Grey coloured, round, gamma hemolytic colonies
S. aureus	7443	Blood agar	Medium to large, smooth, entire, slightly raised, creamy yellow, with green/beta hemolytic colonies
		Nutrient agar	As above without hemolytic activity
A. baumannii	1425	Nutrient agar	Colourless smooth, opaque, raised and pinpoint
		MacConkey agar	Colourless smooth, opaque, raised, NLF
		CLED agar	Blue coloured opaque raised NLF
Citrobacter sp.	1658	MacConkey agar	Late LF light pink after 48h
E. aerogenes	2990	Blood agar	White convex with gamma-hemolysis
		MacConkey agar	LF, mucoid
		Nutrient agar	Flat dry, irregular
		MC agar	LF, flat dry pink, irregular
E. coli	443	EMB agar	Purple coloured, flat dry, irregular colonies, with metallic green colour
		Blood agar	Swarms on blood agar with beta-hemolysis
		CLED agar	Translucent blue
K. pneumoniae	4031	MC agar	LF, pink, mucoid
		CLED agar	Yellow mucoid
K. oxytoca	NA	MacConkey agar	LF, pink, mucoid
		CLED agar	Yellow mucoid
P. mirabilis	NA	MacConkey agar	LLF light pink after 48 h
		Blood agar	Swarms on blood agar with beta-hemolysis
		CLED agar	Translucent blue
P. vulgaris	1771	Blood agar	Swarms on blood agar with beta-hemolysis
		CLED agar	Translucent blue
P. aeruginosa	1688	Nutrient agar	Large, irregular opaque with bluish green pigment

MTCC: Microbial type culture collection; CLED: Cysteine lactose electrolyte deficient; LF: Lactose fermenting; NLF: Non-lactose fermenting; NA: not available.

Table 3

Biochemical identification of the isolated Gram-positive and Gram-negative bacteria.

Bacteria	Catalase	Oxidase	Coagulase	Indole	MR	VP	Citrate	Urease	TSI	Nitrate	Bile esculin
E. faecalis	+	Nd	–	Nd	Nd	Nd	Nd	Nd	Nd	Nd	+
S. aureus	+	Nd	+	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
A. baumannii	+	–	Nd	–	–	+	+	V	nd	–	Nd
Citrobacter sp.	+	–	Nd	–	+	–	+	–	A/A H2S	+	Nd
E. aerogenes	+	–	Nd	–	–	+	+	–	A/A	+	Nd
E. coli	+	–	Nd	+	+	–	–	–	A/AG	+	Nd
K. pneumoniae	+	–	Nd	–	–	+	+	+	A/AG	+	Nd
K. oxytoca	+	–	Nd	+	–	+	+	+	A/A G	+	Nd
P. mirabilis	+	–	Nd	–	+	–	+	+	K/A H2S	+	Nd
P. vulgaris	+	–	Nd	+	+	–	+	+	K/A H2S	+	Nd
P. aeruginosa	+	+	Nd	–	–	–	+	+	Nd	+	Nd

MR: methyl red test; VP: Voges-Proskauer test; TSI: triple sugar iron test; V: variable; A/A: acid in slant and butt; A/AG H_2S: acid in slant and butt with H_2S gas production; A/AG: acid in slant and butt with gas production; K/A H_2S: alkali in slant and butt with H_2S gas production; Nd: not done; +: positive; –: negative.

45% of 62 strains of *P. mirabilis*, 24% to 53% of 47 strains of *P. vulgaris*, 44% to 71% of 80 strains of *P. aeruginosa*. Among these three antibiotics, gentamicin was recorded to be more resistant to these pathogens (Table 4).

Similarly, percentages of resistance patterns of two Gram-positive bacteria with five antibiotics of the beta-lactam group are detailed (Table 5); resistance patterns were in ranges: 51% to 76% of 115 strains of *E. faecalis*, 45% to 85% of 152 strains of *S. aureus*. Likewise, Gram-negative bacteria were tested for four beta-lactams only with resistance patterns as given: 47% to 77% of 74 strains of *A. baumannii*, 24% to 46% of 50 strains of *Citrobacter* sp., 45% to 75% of 72 strains of *E. aerogenes*, 62% to 89% of 194 strains of *E. coli*, 51% to 83% of 108 strains of *K. pneumoniae*, 27% to 46% of 42 strains of *K. oxytoca*, 15% to 45% of 62 strains of *P. mirabilis*, 25% to 48% of 47 strains of *P. vulgaris*, 49% to 77% of 80 strains of *P. aeruginosa*. For Gram-negative bacteria, antibiotics were resistant in the order: amoxyclav>ampicillin>piperacillin/tazobactam> piperacillin. But with Gram-positive bacteria, such an

Table 4

Percentages of resistance of all clinically isolated bacteria to three antibiotics of the aminoglycoside group (%).

Bacteria	Amikacin 30 μg/disc			Gentamicin 10 μg/disc			Netilmicin 30 μg/disc		
	1 st phase	2 nd phase	3 rd phase	1 st phase	2 nd phase	3 rd phase	1 st phase	2 nd phase	3 rd phase
E. faecalis	54	67	65	57	65	76	63	66	71
S. aureus	68	74	79	58	79	85	61	79	75
A. baumannii	47	52	61	62	57	64	47	61	64
Citrobacter sp.	28	31	48	35	39	46	28	32	36
E. aerogenes	65	72	78	52	61	63	65	80	81
E. coli	71	74	81	74	79	78	51	57	58
K. pneumoniae	65	76	79	57	59	53	65	72	77
K. oxytoca	17	27	25	27	34	32	17	25	32
P. mirabilis	27	29	32	29	43	45	27	31	35
P. vulgaris	35	38	34	24	37	53	35	42	44
P. aeruginosa	54	49	67	59	67	71	44	57	61

1st phase: April-September 2011; 2nd phase: October 2011-March 2012; 3rd phase: April-September 2012.

Table 5

Percentages of resistance of all clinical isolated bacteria to five antibiotics of the β-lactam group (%).

Bacteria	Amoxyclav 30 μg/disc			Ampicillin 10 μg/disc			Oxacillin 1 μg/disc			Piperacillin 100 μg/disc			Piperacillin/tazobactam 100/10 μg/disc		
	1 st phase	2 nd phase	3 rd phase	1 st phase	2 nd phase	3 rd phase	1 st phase	2 nd phase	3 rd phase	1 st phase	2 nd phase	3 rd phase	1 st phase	2 nd phase	3 rd phase
E. faecalis	62	67	70	52	68	69	56	61	64	51	57	65	57	65	76
S. aureus	71	77	82	68	74	76	45	58	74	48	54	59	58	79	85
A. baumannii	55	62	67	69	77	59	–	–	–	47	52	51	62	57	64
Citrobacter sp.	25	31	40	32	37	44	–	–	–	25	24	28	33	39	46
E. aerogenes	52	61	72	65	69	75	–	–	–	45	52	61	52	61	63
E. coli	78	82	89	62	71	78	–	–	–	72	78	81	74	79	78
K. pneumoniae	54	67	76	74	79	83	–	–	–	55	61	67	51	59	62
K. oxytoca	35	34	38	33	39	46	–	–	–	27	31	35	37	34	35
P. mirabilis	15	22	31	32	41	43	–	–	–	34	39	42	34	43	45
P. vulgaris	42	38	41	34	39	48	–	–	–	25	34	41	35	37	43
P. aeruginosa	65	71	77	51	54	61	–	–	–	56	69	71	49	61	68

1st phase: April-September 2011; 2nd phase: October 2011-March 2012; 3rd phase: April-September 2012; –: not used, as oxacillin is not used for Gram-negatives.

order would be: amoxyclav>piperacillin/tazobactam>ampicillin>oxacillin>piperacillin (Table 5).

Further, resistance-percent values of UTI-bacteria to cephalosporin antibiotics (cefepime, ceftazidime and cefuroxime) in three 6-month phases were in ranges, 51% to 76% of 115 strains of *E. faecalis*, 61% to 86% of 152 strains of *S. aureus*, 38% to 62% of 74 strains of *A. baumannii*, 22% to 40% of 50 strains of *Citrobacter* sp., 32% to 79% of 72 strains of *E. aerogenes*, 58% to 82% of 194 strains of *E. coli*, 54% to 75% of 108 strains of *K. pneumoniae*, 21% to 47% of 42 strains of *K. oxytoca*, 27% to 37% of 62 strains of *P. mirabilis*, 34% to 47% of 47 strains of *P. vulgaris*, 67% to 83% of 80 strains of *P. aeruginosa* (Table 6). All these three antibiotics were almost equally resistant to the isolated UTI pathogens, confirming the consistence in the production of ESBL by majority of isolates.

Similarly, resistance-percent values of UTI-bacteria to antibiotics of the fluoroquinolone group (gatifloxacin, levofloxacin, norfloxacin and ofloxacin) in three 6-month phases were in ranges, 37% to 72% of 115 strains of *E. faecalis*, 38% to 83% of 152 strains of *S. aureus*, 22% to 81% of 74 strains of *A. baumannii*, 15% to 58% of 50 strains of *Citrobacter* sp., 32% to 76% of 72 strains of *E. aerogenes*, 44% to 90% of 194 strains of *E. coli*, 47% to 87% of 108 strains of *K. pneumoniae*, 21% to 17% of 40 strains of *K. oxytoca*, 19% to 52% of 62 strains of *P. mirabilis*, 24% to 49% of 47 strains of *P. vulgaris*, 37% to 77% of 80 strains of *P. aeruginosa* (Table 7). These antibiotics were resistant to UTI-pathogens in the order: ofloxacin>gatifloxacin>norfloxacin>levofloxacin; the later one was newly introduced.

Lastly, detailed antibiograms of three stand-alone antibiotics, co-trimoxazole, nitrofurantoin, and vancomycin were recorded. Surprisingly, vancomycin 30 μg/disc was found resistance for 27% and 26% of strains of *E. faecalis* and *S. aureus*, respectively in this hospital (Table 8). Nine Gram-negative bacteria were tested for two stand-alone antibiotics with resistance patterns as given: 55% to 67% of 74 strains of *A. baumannii*, 27% to 39% of 50 strains of *Citrobacter* sp., 34% to 62% of 72 strains of *E. aerogenes*, 35% to 71% of 194 strains of *E. coli*, 66% to 78% of 108 strains of *K. pneumoniae*, 22% to 42% of 42 strains of *K. oxytoca*, 27% to 36% of 62 strains of *P. mirabilis*, 22% to 39% of 47 strains of *P. vulgaris*, 63% to 79% of 80 strains of *P. aeruginosa* (Table 8).

Table 6

Percentages of resistance of all clinical isolated bacteria to three antibiotics of the cephalosporin group (%).

Bacteria	Cefepime 30 μg/disc			Ceftazidime 30 μg/disc			Cefuroxime 30 μg/disc		
	1st phase	2nd phase	3rd phase	1st phase	2nd phase	3rd phase	1st phase	2nd phase	3rd phase
E. faecalis	65	70	76	62	68	69	51	54	58
S. aureus	61	67	78	78	84	86	62	66	68
A. baumannii	38	42	49	32	41	48	50	56	62
Citrobacter sp.	29	35	40	22	27	34	37	38	38
E. aerogenes	32	41	52	35	39	45	72	77	79
E. coli	58	62	69	67	71	78	75	80	82
K. pneumoniae	54	67	71	64	69	73	71	72	75
K. oxytoca	35	41	47	21	29	32	32	39	42
P. mirabilis	27	31	35	37	34	35	27	31	34
P. vulgaris	34	39	42	34	43	45	38	46	47
P. aeruginosa	75	79	81	75	77	83	67	76	82

1st phase: April-September 2011; 2nd phase: October 2011-March 2012; 3rd phase: April-September 2012.

Table 7

Percentages of resistance of all clinical isolated bacteria to four antibiotics of the fluoroquinolone group (%).

Bacteria	Gatifloxacin 5 μg/disc			Levofloxacin 5 μg/disc			Norfloxacin 10 μg/disc			Ofloxacin 5 μg/disc		
	1st phase	2nd phase	3rd phase	1st phase	2nd phase	3rd phase	1st phase	2nd phase	3rd phase	1st phase	2nd phase	3rd phase
E. faecalis	44	49	54	37	45	46	–	–	–	61	68	72
S. aureus	69	77	83	38	39	45	–	–	–	64	70	74
A. baumannii	47	56	62	22	37	34	37	41	44	79	81	47
Citrobacter sp.	48	51	58	15	29	29	38	42	56	35	41	37
E. aerogenes	55	61	66	32	41	43	65	70	76	62	68	69
E. coli	82	84	90	44	59	63	61	67	78	78	84	86
K. pneumoniae	75	79	81	47	49	53	75	82	87	69	77	59
K. oxytoca	21	25	31	17	24	27	29	35	40	22	27	34
P. mirabilis	27	32	38	19	23	25	32	41	52	35	39	45
P. vulgaris	31	38	44	24	27	33	38	42	49	32	41	48
P. aeruginosa	64	69	77	59	37	41	54	67	71	64	69	73

1st phase: April-September 2011; 2nd phase: October 2011-March 2012; 3rd phase 3: April-September 2012; –: not used.

Table 8

Percentages of resistance of all clinical isolated bacteria to three stand-alone antibiotics (%).

Bacteria	Co-trimoxazole 25 μg/disc			Nitrofurantoin 300 μg/disc			Vancomycin 30 μg/disc		
	1st phase	2nd phase	3rd phase	1st phase	2nd phase	3rd phase	1st phase	2nd phase	3rd phase
E. faecalis	45	56	64	–	–	–	12	19	27
S. aureus	72	74	79	–	–	–	08	15	26
A. baumannii	55	64	67	58	59	62	–	–	–
Citrobacter sp.	27	35	39	27	34	39	–	–	–
E. aerogenes	54	59	62	34	43	45	–	–	–
E. coli	55	67	71	35	37	43	–	–	–
K. pneumoniae	66	69	78	69	71	76	–	–	–
K. oxytoca	33	35	42	22	29	32	–	–	–
P. mirabilis	27	31	35	28	35	36	–	–	–
P. vulgaris	25	32	39	22	31	33	–	–	–
P. aeruginosa	63	68	72	74	79	78	–	–	–

1st phase: April-September 2011; 2nd phase: October 2011-March 2012; 3rd phase: April-September 2012; –: not used.

4. Discussion

The UTI problem in hospitalized patients could be symptomatic or asymptomatic. The later included potential infections to cause to symptoms later on. Apart from the promotion of UTI from fecal matter, it is more readily in females than that of in males[20,21], catheter-associated UTIs in both males and females are rampant[22,23]. In an Iranian study, the average length hospitalization for symptomatic development of UTI was recoded as 9.96 days[23]; but fewer days, *i.e.*, 7 d were reported with the catheter-associated UTI development elsewhere[24]. In summary, the most

frequently UTI pathogens could be arranged in the decreasing order, *Klebsiella*, *E. coli*, *Pseudomonas* and *Enterobacter*; not surprisingly the fungus *Candida* sp. was also the most common pathogen[25,26].

Resistance to a pathogenic bacterium is mostly determined by the past infection-history of a patient; eventually, resistance to some targeted or non-targeted pathogen might be more likely[27]. Secondly, because of simple and plastic genomes of bacteria, the emergence of resistant mutants for a class of antibiotics due to one used from that class is more likely than imagined, at least for the chance factor. Moreover, it had been elucidated earlier that a drug resistant mutant in a population of 10^6-10^8 cells is most likely, without any involvement of the widely-talked-about R-plasmids, conferring resistance[28]. It is also consensus that mechanisms of drug resistance are multidimensional and there may be the expression of certain genes, beta-lactamase that degrades the applied beta-lactam or cephalosporin antibiotics[29], or carbapenemases, degrading meropenem or imipenem or altered channels in the cell membrane that would disallow antibiotics for the entry into cells of the pathogen. The later mechanism had been demonstrated in *E. coli*[30]. Further, the camaraderie of exchange of genetic matter in bacteria is outlandish in that, in addition to the inter-specific gene transfer within the genus, inter-generic bacterial gene transfer had also been demonstrated via bacterial transformation and conjugation[31]. The transfer of the multiple antibiotic resistance (mar) locus from *E. coli* to the phylogenetically distant *Mycobacterium smegmatis* is a surprising example[32]. During the gene transfer process even, transposons/mass transfer of characters occur, which could, *a priory*, lead to the enrichment of characters of drug resistance in a cell, progressively causing the emergence of bacterial strains, resistant to almost all drugs/antibiotics in current use or pandrug resistant (PDR) bacteria, as they are known[33], to state with a bold conjecture[34]. Genetic exchange mechanisms cause improvements of mutants for further drug-resistance. All pathogenic cells in the body slowly get replaced by a progeny of the resistant cell that act as a doppelgänger, eventually the chicaning MDR strain of a bacterium predominates[34]. Each of the mechanism described are potent enough to afford drug-resistance to any bacteria, may be Gram-negative or Gram-positive and phylogenetically near or distant. The rising rates of the emergence of MDR Gram-negatives, *A. baumannii*, *K. pneumoniae*, and *P. aeruginosa* are regarded as ferocious PDR bacteria[33]. Among Gram-positives, *S. aureus* and *Enterococcus* sp. are notoriously MDR and precipitate fervent episodes[35]. Alike *E. coli*, *S. aureus* was a commensal of soft tissues and internal nares of nose, but methicillin resistant *S. aureus* (MRSA) strains have become resistant to the penicillin group of antibiotics due to *ESBL* genes. Not surprisingly, MRSA has slowly developed resistances to all other antibiotics of all major classes of the time, even many of which were never used for MRSA, raising its survival strength to withstand 23 antibiotics[36]. MRSA has now become MDR-MRSA and is considered as the superbug in the health domain. This bacterium is the primary cause of suppurative infection and creates intimidatory clinical consternations mainly in surgical wound sites[36].

It could be spelled out that infectious diseases are mismanaged by hospitals in almost all countries. A clinician sometimes prescribe an antibiotic of the latest generation to have a dramatically control-effect over the infection[37], but the aftermath is the emergence of one or other MDR bacteria because of the chance factor, discussed herein. There are many resistant strains in the body without any symptom of acute infection in healthy individuals. However, this situation leads to a doggedly condition of a shield favouring to the marauded pathogen causing the failure of any antibiotics on application that had been used from the previously used antibiotic generation. Further, sometime a patient takes the prescribed antibiotic irregularly, probably even stops it after a few dose of intake blithely, due to the control of the infection, but the resistant mutant returns to activity, and burgeons because of the absence of the antibiotic-stress, eventually a population of MDR bacteria spreads in the body. This usually happens in imunocompromised and aged patients frequently. Further, a dose of an antibiotic is fixed at a lower concentration to prevent any non-target adverse effects of antibiotics on the body; such a concentration would be below a host-annoyance causing level-the mutant preventive concentration[38], eventually with an aftermath of bacterial drug resistance, epitomized for tubercle bacillus[38]. Further, both in developed and developing countries[39,40], patients often take medicines without any medical prescription, which would lead to mismatches in giving ways to the development of resistant mutant strains to the employed antibiotics for any non-targeted pathogen/commensal. This could be the conceivable mechanism of transformation of a harmless commensal to a MDR pathogen, just like *S. aureus*. Empirically, basing on symptoms, a clinician often prescribes antibiotics for an unknown viral infection, or a preemptive measure for unwarranted opportunistic bacterial infection, leading to an abuse of antibiotics and promoting the emergence of resistant mutant strains, consequently. Each issue described herein might appear trivial, but their cumulative effects should create a bold conjecture in the development and spread of MDR pathogens everywhere, which have been amply reported[41].

It has been reported that acute UTI cases due to nosocomial occurrence of *E. faecalis* was 35% in a typical study[42]. In another study, 31.4% males and 21.5% females picked up UTI with *Enterococci* sp., because of lower economic status[43]. Moreover, indwelling urinary catheters (IUCs) is a commonly-used medical device in intensive/critical care units of hospitals at least for 10-30 d. In fact, IUC-associated UTI is the second major device associated nosocomial infection. In an American study, about 560000 cases of IUC associated UTI have been recorded[44]. In Indian context, diabetic patients living in urban slums with unhygienic conditions and malnutrition suffer from recurrent infections with multiple UTI-causing organisms[43], despite governmental remedial measures on child and women welfare.

In conclusion, antibiotic sensitivity patterns of 11 UTI-causing bacteria recorded in this study indicated moderately higher numbers of strains resistant to each antibiotic studied. Both *S. aureus* and *E. faecalis* were vancomycin resistant and resistant-strains increased in each 6-month period of study. All Gram-negatives were resistant to nitrofurantoin and co-trimoxazole, the most preferred antibiotics of empiric therapy for UTI. If suitable control measures are not taken up, the cohort of iconic UTI-organisms, *A. baumannii*, *E. coli*, *K. pneumoniae*, and *S. aureus* could precipitate fervent episodes in public health, as these are classified as cataclysmic PDR bacteria. Viewed from the trenches of public health, spread of MDR pathogens could be the cause of loss of hygienic totem pole of any countries-may be developed or developing, developed probably due to the absence of a stringent

antibiotic policy, and additionally, could be partly attributed to the intransigent attitude of both medical and paramedical staff to the well-known, impeccable antiseptic measures, during the management of catheter insertions, prevention of needle-stick injuries, scientific disposal of hospital sewage and a few more. Obviously, the absence of hygienic awareness in urban-slum dwellers and public also adds fuel to fire of the problem of the subtle spread of MDR pathogens; and in communities MDR pathogens should aslo hurtle, *a priory*. In developing countries as seen India, a disproportionately large mass of patients and their attendants, attending hospitals during prognosis and treatments could escalate the spread of MDR pathogens in nosocomial settings. Indeed, eyebrow-raising values of antibiotic resistance of the whole gamut of UTI pathogens as recorded herein, for all most all antibiotics in use at the time, are of clinical consternation for patients from more than half of human population. At such a beleaguered pandemonium, leaning to complementary and alternative medicines would be a prudent and pragmatic approach. Obviously, recourse to plants could provide avant-garde drugs, as those are widely held today by many developed and the most developing countries with directives of World Health Organization. Floccinaucinihilipilification of phyto-drugs is now regarded as a pejorative attitude.

Conflict of interest statement

The authors declare that they have no conflict of interests.

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Comments

Background

About a 35% of UTI infection are of nosocomial origin. This problem has been magnified over the time with the emergence of multidrug resistant bacteria and it has become a frequently met with medical problem. The transformation of the commensal, *E. coli* mostly isolated from patients with uncomplicated, as a notorious pathogen is of utmost consternation, for example. Further, its strains gained the capability of the production of extended spectrum beta-lactamase (*ESBL*) enzyme, capable of degrading antibiotics of beta-lactam and cephalosporin groups. Further, several other Gram-negative notorious UTI-bacteria are *A. baumannii*, *P. aeruginosa*, *Proteus* sp., *Klebsiella* sp., *C. trachomatis* and *N. gonorrhea*, mainly.

Research frontiers

This study records infection of a vast majority on MDR bacteria, it has become a matter of compulsion to conduct a regional surveillance on this exasperating class of pathogens, causing morbidity and mortality in females mainly.

Related reports

It has been reported that acute UTI cases due to nosocomial occurrence of *E. faecalis* was 35% (Saleem and Daniel 2011). In another study, 31.4% males and 21.5% females picked up UTI with *Enterococci* sp., because of lower economic status (Chen *et al*. 2009). Moreover, indwelling urinary catheters (IUCs) is a commonly-used medical device in intensive/critical care units of hospitals, at least for 10-30 d. In fact, IUC-associated UTI is the second major device associated nosocomial infection. In an American study, about 560000 cases of IUC associated UTI have been recorded (Newman 2011). A study from Tamil Nadu, India recorded predominance of bacteria as follows: *E. coli* (31.5%), *S. aureus* (20.5%), *K. pneumoniae* (15.8%), *P. aeruginosa* (7.5%) and *Proteus* sp. (7.4%); their strains were resistant to antibiotics (μg/disc) at decreasing levels: trimethoprim- sulphamethaxazole-30 (83.3%), nalidixic acid-30 (67.3%), amoxicillin-10 (67.3%), co-trimoxazole-10 (61%), gentamicin-10 (48.8%), ciprofloxacin-10 (46%) and cefotaxime-10 (43%), *in vitro* (Manikandan *et al*. 2011).

Innovations and breakthroughs

Data on antibiotic sensitivity patterns of 11 UTI-causing bacteria recorded in this study indicated moderately higher numbers of strains resistant to each antibiotic studied. Both *S. aureus* and *E. faecalis* were vancomycin resistant and resistant-strains increased in each 6-month period of study. All Gram-negatives were resistant to nitrofurantoin and co-trimoxazole, the most preferred antibiotics of empiric therapy for UTI.

Applications

MDR pathogens could be the cause of the absence of a stringent antibiotic policy, and additionally, could be partly attributed to the intransigent attitude of both medical and paramedical staff to the well-known, impeccable antiseptic measures, during the management of catheter insertions, prevention of needle-stick injuries, scientific disposal of hospital sewage and a few more. We could be conscious on these aspects.

Peer review

This is a good study, in which the authors evaluated antibiogam of notorious UTI causing bacteria isolated from clinical samples of a hospital, for a dreadful disease. Antibiograms of bacteria indicated moderately higher numbers of strains resistant to each antibiotic studied, generating the fear of precipitating fervent episodes in public health particularly with bacteria.

References

[1] Drekonja DM, Johnson JR. Urinary tract infections. *Prim Care* 2008; **35**: 345-367.
[2] Taneja N, Chatterjee SS, Singh M, Singh S, Sharma M. Pediatric urinary tract infections in a tertiary care center from Northern India. *Indian J Med Res* 2010; **131**: 101-105.
[3] Jeyaseelan EC, Jashothan PT. *In vitro* control of *Staphylococcus aureus* (NCTC 6571) and *Escherichia coli* (ATCC 25922) by *Ricinus communis* L. *Asian Pac J Trop Biomed* 2012; **2**(9): 717-721.
[4] Behzadi P, Behzadi E, Yazdanbod H. Urinary tract infections associated with *Candida albicans*. *Maedica* 2010; **5**: 277-279.
[5] Kawecki D, Kwiatkowski A, Sawicka-Grzelak A, Durlik M, Paczek L, Chmura A, et al. Urinary tract infections in the early post transplant period after kidney transplantation: etiologic agents and their

susceptibility. *Transplant Proc* 2011; **43**: 2991-2993.
[6] Moreno E, Andreu A, Pigrau C, Kuskowski MA, Johnson JR, Prats G. Relationship between *Escherichia coli* strains causing acute cystitis in women and the fecal *E. coli* population of the host. *J Clin Microbiol* 2008; **46**: 2529-2534.
[7] Manikandan S, Ganesapandian S, Singh M, Kumaraguru AK. Emerging of multidrug resistance human pathogens from urinary tract infections. *Curr Res Bacteriol* 2011; **4**: 9-15.
[8] Abrahamian FM, Moran GJ, Talan DA. Urinary tract infections in the emergency department. *Infect Dis Clin North Am* 2008; **22**: 73-87.
[9] Lane DR, Takhar SS. Diagnosis and management of urinary tract infection and pyelonephritis. *Emerg Med Clin North Am* 2011; **29**: 539-552.
[10] D'Andrea MM, Venturelli C, Tommaso Giani T, Fabio Arena F, Viola C, Paola B, et al. Persistent carriage and infection by multidrug-resistant *Escherichia coli* ST405 producing NDM-1 carbapenemase: Report on the first Italian cases. *J Clin Microbiol* 2011; **49**: 2755-2758.
[11] Bours PHA, Polak R, Hoepelman AIM, Delgado E, Jarquin A, Matute AJ. Increasing resistance in community-acquired urinary tract infections in Latin America, five years after the implementation of national therapeutic guidelines. *Int J Infect Dis* 2010; **14**: e770-e774.
[12] Karlowsky JA, Lagace-Wiens PR, Simner PJ, DeCorby MR, Adam HJ, Walkty A, et al. Antimicrobial resistance in urinary tract pathogens in Canada from 2007 to 2009: CANWARD surveillance study. *Antimicrob Agents Chemother* 2011; **55**: 3169-3175.
[13] Perrotta C, Aznar M, Mejia R, Albert X, Ng CW. Oestrogens for preventing recurrent urinary tract infection in postmenopausal women. *Obstet Gynecol* 2008; **112**(3): 689-690.
[14] Geerlings SE. Urinary tract infections in patients with diabetes mellitus: epidemiology, pathogenesis and treatment. *Int J Antimicrob Agents* 2008; **31**: S54-S57.
[15] Baishya RK, Mathew A, Dhawan DR, Desai MR. Urinary tract infection in diabetic patients. *Indian J Nephrol* 2010; **20**: 222.
[16] Sahu MC, Rath S, Dubey D, Debata NK, Padhy RN. Multidrug resistance of *Pseudomonas aeruginosa* as known from surveillance of nosocomial and community infections in an Indian teaching hospital. *J Publ Health* 2012; **20**: 413-423.
[17] Orsi GB, Falcone M, Venditti M. Surveillance and management of multidrug-resistant microorganisms. *Expert Rev Anti Infect Ther* 2011; **9**: 653-679.
[18] Forbes BA, Sahm DF, Weissfeld AS. *Bailey and Scott's diagnostic microbiology*. 12 th ed. St. Louis: Mosby Elsevier; 2007.
[19] Clinical and Laboratory Standards Institute. Performance standard for antimicrobial susceptibility testing: twenty-first informational supplement. Document M200-S21. Wayne, PA: CLSI; 2011.
[20] Iosifidis E, Antachopoulos C, Tsivitanidou M, Katragkou A, Farmaki E, Tsiakou M, et al. Differential correlation between rates of antimicrobial drug consumption and prevalence of antimicrobial resistance in a tertiary care hospital in Greece. *Infect Control Hosp Epidemiol* 2008; **29**: 615-622.
[21] Hsu LY, Tan TY, Tam VH, Kwa A, Fisher DA, Koh TH, et al. Surveillance and correlation of antibiotic prescription and resistance of Gram-negative bacteria in Singaporean hospitals. *Antimicrob Agents Chemother* 2010; **54**: 1173-1178.
[22] Wagenlehner FM, Wagenlehner C, Savov O, Gualco L, Schito G, Naber KG. Clinical aspects and epidemiology of uncomplicated cystitis in women—German results of the ARESC study. *Urologe A* 2010; **49**: 253-261.
[23] Taher MT, Golestanpour A. Symptomatic nosocomial urinary tract infection in ICU patients: identification of antimicrobial resistance pattern. *Iranian J Clin Infect Dis* 2009; **4**: 25-29.
[24] Gupta A, Sharma S, Arora A, Gupta A. Changing trends of *in vitro* antimicrobial resistance patterns in blood isolates in a tertiary care hospital over a period of 4 years. *Indian J Med Sci* 2010; **64**: 485-492.
[25] Jaggi N, Sissodia P, Sharma L. Control of multidrug resistant bacteria in a tertiary care hospital in India. *Antimicrob Resist Infect Control* 2012; **1**: 23.
[26] Chopra I, Schofield C, Everett M, O'Neill A, Miller K, Wilcox M, et al. Treatment of health-care-associated infections caused by Gram-negative bacteria: A consensus statement. *Lancet Infect Dis* 2008; **8**: 133-139.
[27] Jernberg C, Löfmark S, Edlund C, Jansson JK. Long-term impacts of antibiotic exposure on the human intestinal microbiota. *Microbiol* 2010; **156**: 3216-3223.
[28] Gillespie SH, Basu S, Dickens AL, O'Sullivan DM, McHugh TD. Effect of subinhibitory concentrations of ciprofloxacin on *Mycobacterium fortuitum* mutation rates. *J Antimicrob Chemother* 2005: **56**; 344-348.
[29] Drieux L, Brossier F, Sougakoff W, Jarlier V. Phenotypic detection of extended-spectrum β-lactamase production in Enterobacteriaceae: review and bench guide. *Clin Microbiol and Infect* 2008; **14**: 90-103.
[30] McMurry LM, Levy SB. The periplasmic protein MppA is not involved in regulation of *marA* in *Escherichia coli*. *Antimicrob Agents Chemother* 2011; **55**: 4939.
[31] Bader MS, Hawboldt J, Brooks A. Management of complicated urinary tract infections in the era of antimicrobial resistance. *Postgrad Med* 2010; **122**: 7-15.
[32] Dubey D, Rath S, Sahu MC, Debata NK, Padhy RN. Antimicrobials of plant origin against multi-drug resistant bacteria including the TB bacterium and economics of plant-drugs—Introspection. *Indian J Trad Knowl* 2012; **11**: 225-233.
[33] Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiol Infect* 2012; **18**(3): 268-281.
[34] Dubey D, Rath S, Sahu MC, Debata NK, Padhy RN. Status of multidrug resistance in tubercle bacillus and phytochemicals for the control. *J Publ Health* 2013; **21**(1): 115-119.
[35] Eliopoulos GM. Microbiology of drugs for treating multiple drug-resistant Gram-positive bacteria. *J Infect* 2009; **59**: S17-S24.
[36] Dubey D, Rath S, Sahu MC, Pattnaik L, Debata NK, Padhy RN. Surveillance of infection status of drug resistant *Staphylococcus aureus* in an Indian teaching hospital. *Asian PacJ Trop Dis* 2013; **3**(2): 101-110.
[37] Briceno DF, Correa A, Valencia C, Torres JA, Pacheco R, Montealegre MC, et al. Antimicrobial resistance of Gram-negative bacilli isolated from tertiary-care hospitals in Colombia. *Biomedica* 2010; **30**: 371-381.
[38] Ferrari R, Magnani M, Souza RB, Tognim MCB, Oliveira TCRM. Mutant prevention concentration (MPC) of ciprofloxacin against *Salmonella enterica* of epidemic and poultry origin. *Curr Microbiol* 2011; **62**: 628-632.
[39] Sood S, Gupta R. Antibiotic resistance pattern of community acquired uropathogens at a tertiary care hospital in Jaipur, Rajasthan. *Indian J Community Med* 2012; **37**: 39-44.
[40] Espiritu C. Treatment and prevention of ocular bacterial infections in Asia part II: the changing landscape of antibiotic treatment. *Asian J Ophthalmol* 2008; **10**: 388-394.
[41] Khan AU, Raffaele Z. Multidrug resistance: A global concern. USA: Bentham Publishers; 2012.
[42] Saleem M, Daniel B. Prevalence of urinary tract infection among patients with diabetes in Bangalore city. *Int J Emerg Sci* 2011; **1**: 133-142.
[43] Chen SL, Jackson SL, Boyko EJ. Diabetes mellitus and urinary tract infection: Epidemiology, pathogenesis and proposed studies in animal models. *J Urol* 2009; **182**: S51-S56.
[44] Newman DK. Prevention and management of catheter-associated UTIs. *Infect Dis* 2010; **Spl Edn**: 13-20.