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Surveillance of ESBL producing multidrug resistant *Escherichia coli* in a teaching hospital in India

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PEER REVIEW

ABSTRACT

Objective: To record nosocomial and community−acquired accounts of antibiotic resistance in *Escherichia coli* (*E. coli*) strains, isolated from clinical samples of a teaching hospital by surveillance, over a period of 39 months (November 2009−January 2013).
Methods: Clinical samples from nosocomial sources, *i.e.*, wards and cabins, intensive care unit (ICU) and neonatal intensive care unit (NICU), and community (outpatient department, OPD) sources of the hospital, were used for isolating strains of *E. coli*, which were subjected for testing for production of 'extended spectrum beta−lactamase'−(ESBL) enzyme as well as determining antibiotic sensitivity pattern with 23 antibiotics.
Results: Of the total 1642 (100%) isolates, 810 (49.33%) strains were from OPD and 832 (50.66%) were from hospital settings. Occurrence of infectious *E. coli* strains increased in a mathematical progression in community sources, but in nosocomial infections, such values remained almost constant in each quarter. A total of 395 (24.05%) ESBL strains were isolated from the total 810 isolates of community; of the total of 464 (28.25%) isolates of wards and cabins, 199 (12.11%) were ESBL strains; and among the total of 368 (22.41%) isolates of ICU and NICU, ESBLs were 170 (10.35%); the total nosocomial ESBL isolates, 369 (22.47%) were from the nosocomial total of 832 (50.66%) isolates. Statistically, it was confirmed that ESBL strains were equally distributed in community or hospital units. Antibiogram of 23 antibiotics revealed progressive increases of drug−resistance against each antibiotic with the maximum resistance values were recorded against gentamicin: 92% and 79%, oxacillin: 94% and 69%, ceftriaxone: 85% and 58%, and norfloxacin 97% and 69% resistance, in nosocomial and community isolates, respectively.
Conclusions: This study revealed the daunting state of occurrence of multidrug resistant *E. coli* and its infection dynamics in both community and hospital settings.

KEYWORDS
E. coli, Nosocomial, ESBL strains, Multidrug−resistance, Community, Infection dynamics

1. Introduction

Several strains of *Escherichia coli* (*E. coli*) are quite harmless and colonize the healthy human bowel, but recently developed strains are pathogenic causing frequently, cholecystitis, bacteremia, cholangitis, urinary tract infections (UTI), traveler's diarrhoea, neonatal meningitis, pneumonia, liver abscess and a few more. Among these diseases, enteric infection are more prevalent and, virulent *E. coli* strains including the strain, 0157:H7 cause gastroenteritis and the normal food poisoning; the second most prevalent strains cause UTI. Rarely, hemolytic uremic syndrome and thrombocytopenic purpura caused by *E. coli* are too known[1]. Overall, it is regarded as the most frequently occurring Gram−negative pathogen today.

For the control of *E. coli* infections, aminoglycosides, β−lactam antibiotics, cephalosporins and fluoroquinolones are often used. In a natural course, this pathogen slowly has developed strains independently resistant to several antibiotics

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of these groups, among which strains with the stratagem of the production of the extended spectrum β−lactamase (ESBL) enzyme that help degrade the β−lactam moiety of penicillin derivatives, cephalosporins, monobactams, and carbapenems spearhead[2]. Consequently, E. coli strains resistant to antibiotics of other groups along with β−lactams, concomitantly emerge, conferring an armamentarium of multiple resistances, that help to burgeon as pathogen and cause consternations in clinical managements.

Further, ESBL−producing E. coli strains were collected since last three decades from hospitalized patients[2]. Moreover, infections caused by an avalanche of pugnacious Enterobacteriaceae−genera producing ESBL have been described from hospitals/extended−care facilities with increasing frequencies[3], creating therapeutic problems, which could be evaded now by the newer cephamycins and carbapenems. And multidrug resistant (MDR) transporters that expel drugs from the cytoplasm or cytoplasmic membrane to the external medium constitute one of the proven causes of arrival of MDR bacteria, as demonstrated in E. coli[4]. Camaraderie in bacteria (both pathogenic and non−pathogenic) help exchange of genetic materials in aggrandizement of multidrug resistance, from one another[3], eventually landing at intractable clinical managements with bacterial pathogens[5]. Cataclysmic rather staggering emergence of pandrug resistance (resistance to all of antibiotics in use) is recorded in E. coli, Pseudomonas aeruginosa, Acinetobacter baumannii and Klebsiella pneumoniae[6], that mire patients to a spate of comorbidities, as if living in a hospice, challenging the clinical or rather cleanly totem pole of a hospital. Specifically, diarrhoea is documented as the second determinative cause of death among under−5− children, globally; nearly one in five child deaths−about 1.5 million deaths each year is due to diarrhoea; mortality rates are estimated to be more in young children than AIDS, malaria and measles combined[7]. The under−5 mortality figures due to diarrhoea of South−East Asian countries was second to that of Africa[8].

Indeed, MDR pathogens increase the overall vulnerability of human health particularly in India[9], with a smattering of well−heeled mass and disproportionately large mass of marginalized people, the rise of clinical costs can never be left as an unheeded concern in public health; secondly, the child mortality must be minimized. Failure in the control of MDR strains of E. coli, Pseudomonas aeruginosa and Staphylococcus aureus, for example, cannot be ordinarily appropriated in the light of logistic principles of public health that would land at pandemic spread and it would be a medical misdemeanor, otherwise. Exemplary sporadic paroxysmal outbreaks of enteropathogenic diseases due to MDR strains of E. coli, Klebsiella sp., Salmonella sp. and Vibrio cholerae have been frequently confronted in tribal Odisha distraughtly, in the last decade[10]. A bacterium often picks some resistance− character from related and even unrelated bacteria intrinsically. Paradigmatically, E. coli strains from pet dogs are phylogenetically similar to pathogenic E. coli strains in human beings; and more than 15% of general canine fecal deposits

in the environment contain E. coli strains related to virulent human strains confirming the zoonosis[11]. There is also a consensus that colossal uses of same antibiotics in maintenance of health of man, pet and food animals including cattle and a few more (veterinary), as well as in agriculture are supposed to contribute significantly to the problem drug resistance in bacteria[12]. Further more, drug−resistance characters in pathogenic strains, achieved through mechanisms of genetic recombination naturally are increasingly of commonplace[13], apart from mutations with selection pressure from the presence of a specific antibiotic[14], for which resistant strains develop.

Judged in this light, nosocomial infections could be attributed in a considerable part to the indurate attitude or lack of specific awareness among medical staff, and community infections to the lack of general awareness among public too. However, when local factors of nosocomial and community infections would be identified and quantified under effective medical surveillance attempts, a general awareness could be spread among all concerned. It is consensus that, patients with old−age troubles admitted to hospitals for one or the other ailment generally are multi−morbid and have reduced immune−competence, eventually are increasingly compromising to antibiotic resistant bacteria. Indeed, the management of E. coli infection is utterly difficult, because of the observed multiple resistances to several groups of antibiotics[15]. Further more, MDR bacterial strains with artifices of exchange of genetic materials of several pathogens along with E. coli challenge the most coveted well−being of man. Each government, may be of developing or developed nations tries to have the totem pole of well−being of public, but it is easier claimed volubly than taken care of religiously by all governments always, in instituting programmes such as, Public Health Interventions, National Institute for Health and Clinical Excellence, National Horizon Scanning Centre, or public, voluntary, community stakeholders and private philanthropists, as found in England and the US[16].

In fact, from surgical and UTI sites, 27.5% strains were E. coli of 160 bacterial isolates; antimicrobial susceptibility of those E. coli isolates were for ampicillin (80%) and tetracycline (82%), reported from Nigeria[17]. Nosocomial isolates of this pathogen from London hospitals revealed that isolates were resistant to ampicillin (55%) and trimethoprim (45%), and ESBL strains were 5.7% from community and 27.6% from nosocomial sources[18]. A study from South India records 202 ESBL detections in urinary tract swabs and their antibiogram for 9 antibiotics[19]. Another report from New Delhi records multidrug resistance to 11 antibiotics in 26 E. coli strains isolated from blood samples[20].

This study gives systematic information on 1642 strains of E. coli, isolated from different clinical samples and their pedantic antibiogram for 23 antibiotics elucidating rather epitomizing the daunting infection−dynamics of MDR E. coli, by surveillance in a state in central India. Resistance of E. coli to a large number of antibiotics was never reported. Results recorded here revealed that E. coli was more drug resistant than those from London. This work depicts the prevalence of E. coli in slums and rural settings as community acquired accounts. This study

is anticipated to aid information to the apothecary for finesse in instituting congruous antibiotics to combat the chicaning MDR *E. coli* strains.

2. Materials and methods

2.1. Isolation and biochemical identification of E. coli

Clinical samples were collected from outpatient department (OPD) or community, as well as from wards, cabins, intensive care unit (ICU) and neonatal intensive care unit (NICU) of Sum Hospital, for isolation of *E. coli*. Of the total 12 846 clinical samples obtained over a period of 39 months (November 2009–January 2013), only 7 194 samples yielded bacteria. Gram–negative bacteria were cultured on MacConkey agar and 'cysteine deoxycholate electrolyte deficient' (CLED) agar (HiMedia, Mumbai). When on MacConkey agar, medium–sized colonies with bright pink colour (due to inherent lactose fermentation), and on CLED agar yellow coloured, round and large colonies were seen, those were taken to be *E. coli*; and the strain of *E. coli*, Microbial Type Culture Collection strain number 443 (β–lactamase negative) was used as the reference strain for all experiments.

The following biochemical tests were done in succession for the standard strain and clinical isolates of *E. coli*, simultaneously: catalase, oxidase, indole, methyl red, Voges–Proskauer, citrate, urease, triple sugar iron and nitrate reduction tests. Catalase, indole, methyl red and nitrate tests were positive, whereas oxidase, Voges–Proskauer, citrate and urease tests were negative. In triple sugar iron test, only acid but no gas was formed. Sugars, glucose, mannitol and maltose were fermented with the production of both acids and gas. From these results, the isolates were taken as *E. coli*[21]. For further confirmation, these isolates were grown in same media (MacConkey agar and CLED), and on eosin–methylene blue agar, wherein, the colonies had metallic green sheen (Figure 1).
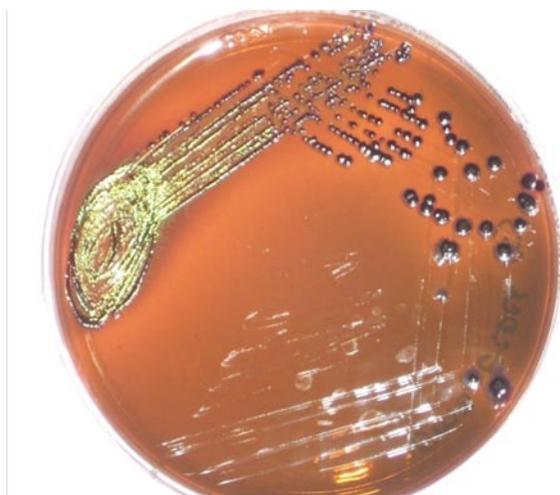

Figure 1. *Escherichia coli* colonies on eosin–methylene blue agar. Green metallic sheen of colonies was evident.

2.2. Detection of ESBL producers by the double disc synergy test

The double disc synergy test (DDST) was used to detect the ESBL activity of selected strains of the resistant *E. coli* isolate on a lawn culture on a Muller–Hinton (MH) agar plate. The augmentin 30 μg disc (20 μg amoxicillin and 10 μg clavulanic acid) in the middle was flanked by a disc of ceftazidime 30 μg and a disc of cefotaxime 30 μg (both third generation cephalosporins), at 30 mm apart on a lawn culture. The set up was done in triplicate and was incubated at 37° C for overnight for observation of inhibition zones. Indeed, the augmentin disc inhibits the action of the ESBL enzyme; eventually, an inhibition zone is formed from a peripheral disc towards the middle, due to the synergistic action of the augmentin and the corresponding cephalosporin disc (Figure 2). In general, if the organism is resistant to both cephalosporins, because of the production of the ESBL enzyme, the action of augmentin deactivates the enzyme with a consequent reactivation of a cephalosporin resulting in the extension of the inhibition zone.

Figure 2. ESBL producing *E. coli* on MHA detected by DDST test (see text for explanation).

For a confirmation and quantitative analysis of ESBL strains, a 96–well micro–titer plate was used to determine minimum inhibitory concentration (MIC) values of two antibiotics of the third generation of cephalosporin, cefotaxime and ceftazidime in broth culture, for 100 selected MDR *E. coli* strains, of which 50 were ESBL producers and 50 were non–ESBLs. An exponential culture of a test strain of *E. coli* in MH broth (HiMedia) was suitably diluted with the normal saline solution to obtain the level of equivalent to the 0.5 McFarland standards. 2,3,5–triphenyltetrazolium chloride, the redox indicator was used as an indicator of bacterial growth. To an aliquot of 20 μL overnight grown test culture, an aliquot of 100 μL of an antibiotic (cefotaxime or ceftazidime) stock solution of 1024 μg/mL and 0.1 mL of MH broth was added to the second well of the micro–titer plate. These solutions were serially diluted in two–fold dilutions at each adjacent well until the final concentration of 0.25 μg/mL antibiotic in the 12th, the last well was obtained. Finally, 0.5% of 5 μL triphenyltetrazolium chloride was added to all wells and the micro–titer plate was incubated at 37 ℃ for 18 h. The wells

were examined for the development of pink colour that in a well indicated the bacterial growth as the absence of the pink colour was taken as the growth inhibition that was recorded as the MIC value[22]. Obviously, the first well of the micro–titer plate was the control without any antibiotic solution. Results were interpreted basing upon the revised break–point values of the third generation cephalosporin antibiotics, according to Clinical and Laboratory Standards Institute guidelines[23]. The reference strain and all the isolated *E. coli* strains were subjected to antibiotic sensitivity tests, by the Kirby–Bauer's method using MH agar, the disc diffusion method, as detailed previously[9].

3. Results

3.1. Occurrence of pathogenic E. coli strains

In this study, the number of *E. coli* isolates from community infections was 810 (49.33%) among the total 1642 (100%) isolates, but the sum of individual nosocomial (wards and cabins 464, and ICU and NICU 368, strains), the percentage was 50.66% (Figure 3). It was evident that the number of pathogenic strains increased in a mathematical progression in community–acquired infections in 39 months. Nevertheless, in nosocomial infections, such values remained almost constant in each quarter in the study period. It was found that *E. coli* was getting uniformly spread in each of the units of the hospital, as in each quarter around 50 cases (from 42 to 64) of pathogenic *E. coli* strains were detected from samples from nosocomial sources. However, there was an increase in incidence of *E. coli* infections in OPD patients (48 to 80) (Tables 1 and 2), which indicated that any patient admitted in to an indoor unit for any ailment, recovers and returns to community with some cryptic infection of a pathogen. Thus, there was a steady increase of incidences of *E.*

coli in patients from community.

Table 1
Isolation of *E. coli* strains from different clinical samples from community (OPD) patients.

Quarter	Samples from community patients					
	Urine	Swabs	Pus	Body fluids	Blood	Total samples
1st	24(15)	13(3)	6(−)	3(−)	2(−)	48(18)
2nd	35(21)	17(8)	7(2)	3(−)	−	62(31)
3rd	42(18)	19(8)	11(3)	4(−)	3(−)	79(29)
4th	37(20)	16(6)	9(1)	2(−)	2(−)	66(27)
5th	35(24)	17(10)	12(2)	4(−)	3(−)	71(36)
6th	43(23)	17(8)	13(3)	6(−)	1(−)	80(34)
7th	28(17)	12(11)	6(1)	4(1)	−	50(30)
8th	33(21)	17(4)	4(1)	3(2)	2(1)	59(29)
9th	31(26)	23(8)	5(−)	4(2)	−	63(36)
10th	27(22)	16(5)	4(2)	3(−)	2(−)	52(29)
11th	33(19)	18(12)	3(−)	2(−)	4(−)	60(31)
12th	32(16)	21(7)	6(3)	3(1)	2(1)	64(28)
13th	26(21)	14(8)	13(7)	2(−)	1(1)	56(37)
Total	426(263)	220(98)	99(25)	43(6)	22(3)	810(395)

1st quarter, Nov 2009–Jan 2010; 13th quarter, Nov 2012–Jan 2013. OPD, outpatient department. Numbers in parenthesis were ESBL *E. coli* strains. Community total isolates, 810 with 395 ESBL isolates.

Among all the reported types of clinical samples from patients of community, urine and swab lots contributed significant numbers of pathogenic *E. coli* strains in each quarter in the reported span of time. Out of the total 810 community isolates, 426 were from urine, 220 were from swabs, 99 strains were from pus samples, 43 strains were from body fluids and 22 strains were from blood samples in 39 months (Table 1). As evident from Table 2, ward and cabins contributed 464 *E. coli* strains. Of them, 216 were from urine, 141 were from swabs and 63 from pus samples. Similarly, patients of ICU and NICU contributed 368 strains of *E. coli* from which, 125 strains were from urine, 104 from swabs, and 46 from pus. Thus, it had been observed

Table 2
Occurrence of *E. coli* strains in different clinical samples from nosocomial patients.

Quarter	Samples from wards and cabins patients						Samples from ICU and NICU patients					
	Urine	Swabs	Pus	Bodyfluids	Blood	Total samples	Urine	Swabs	Pus	Body fluids	Blood	Total samples
1st	17(9)	10(1)	4(1)	1(−)	1(−)	33(11)	7(4)	4(1)	3(2)	2(1)	1(−)	17(8)
2nd	13(12)	11(5)	6(2)	3(−)	2(−)	35(19)	15(3)	9(2)	3(−)	1(−)	1(−)	29(5)
3rd	15(10)	8(4)	5(2)	3(−)	−	31(16)	13(8)	5(4)	4(2)	−	−	22(14)
4th	13(5)	7(5)	4(2)	2(−)	1(−)	27(12)	6(5)	4(4)	3(−)	2(−)	−	15(9)
5th	20(9)	9(9)	2(1)	1(−)	1(−)	33(19)	8(8)	3(−)	2(2)	4(−)	2(−)	19(10)
6th	17(9)	10(6)	3(1)	1(−)	1(−)	32(16)	11(7)	6(5)	3(2)	2(1)	−	22(15)
7th	15(5)	12(3)	4(1)	2(1)	1(−)	34(10)	13(5)	7(5)	4(2)	2(−)	1(−)	27(12)
8th	20(13)	8(2)	7(2)	4(1)	1(−)	40(18)	20(9)	9(9)	2(1)	2(1)	1(−)	34(20)
9th	15(7)	14(3)	4(4)	1(−)	1(1)	35(15)	17(9)	10(6)	3(1)	1(1)	1(−)	32(17)
10th	23(16)	12(−)	4(1)	2(−)	2(−)	43(17)	15(5)	12(3)	4(1)	2(1)	1(−)	34(10)
11th	12(5)	12(4)	3(−)	3(−)	−	30(9)	20(13)	12(4)	5(2)	3(−)	−	40(19)
12th	21(12)	15(3)	9(5)	2(1)	3(1)	50(22)	15(7)	11(5)	6(2)	3(−)	1(1)	36(15)
13th	15(3)	13(8)	8(3)	5(1)	−	41(15)	20(11)	12(3)	4(−)	2(1)	3(1)	41(16)
Total	216(115)	141(53)	63(25)	30(4)	14(2)	464(199)	180(94)	104(51)	46(17)	26(6)	12(2)	368(170)

1st quarter: Nov 2009–Jan 2010; 13th quarter, Nov 2012–Jan 2013. ICU, intensive care unit; NICU, neonatal intensive care unit. Numbers in parenthesis were ESBL *E. coli* strain. Nosocomial total isolates, 832 with 369 ESBL isolates.

that nosocomial sources of samples had maximum numbers of *E. coli* in urine and their preponderance was as follows: urine >swabs>pus>body fluids > blood, in both community and nosocomial sources (Table 2). Thus, *E. coli* is the dominating pathogen spreading UTI, both nosocomial and community sectors.
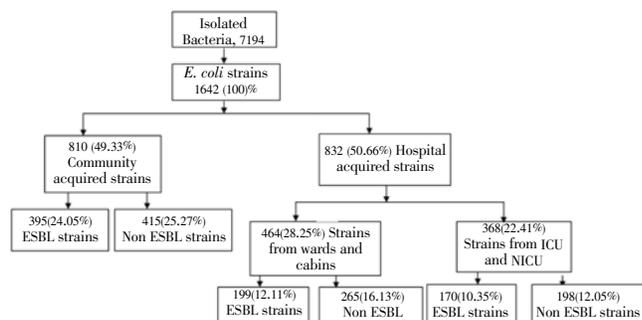

Figure 3. Isolation of *E. coli* strains from different sources.

3.2. ESBL strains

In both community and nosocomial sources, occurrence of ESBL *E. coli* strains were between 40 and 60% in each individual source (Tables 1 and 2). It was found that ESBL isolates were 395 (48.76%, see Figure 3) of total 810 isolates of OPD. Likewise, in wards and cabins, ESBL isolates were 199 of the total of 464 isolates. Of 368 isolates of ICU and NICU those were 170; the total nosocomial ESBL isolates were 369, in this study from the total 832 *E. coli* isolates. The χ^2–test of independence was used to test whether ESBL strains were fractionally more distributed than all other antibiotic–resistant strains in community or wards and cabins or ICU and NICU sources (Tables 1 and 2), using a r×c contingency table. It was found that the computed χ^2–value was 2.172; the tabulated χ^2–value is 5.991, for degree of freedom=2, *P*=0.05; so, the null hypothesis was not rejected, confirming ESBL strains were equally distributed in community or wards

and cabins, or ICU and NICU sources, like the rest other drug–resistant *E. coli* strains.

With a cohort of 50 ESBL producing strains, it was found that when cefotaxime was used, the MIC range was 64 to 256 µg/mL for 24 strains and the rest 26 strains had MIC values of ≥512 µg/mL; similarly, for ceftazidime, the MIC range was 32 to 256 µg/mL for 32 strains and, the rest 18 strains had MIC values of ≥512 µg/mL. These MIC values confirmed the presence of ESBL producing strains, as the break points for cefotaxime and ceftazidime were ≥16 and ≥4 µg/mL, respectively. Similarly, all the 50 non–ESBL producers had a MIC range, 0.5 to 4.0 µg/mL, for both cefotaxime and ceftazidime. Thus, these 50 strains were confirmed to be susceptible to both the antibiotics (Table 3). These results corroborate the results obtained through DDST.

Table 3
Detection of MIC values in ESBL producers with two third generation cephalosporins used in DDST.

Breakpoint (µg/mL)	Cefotaxime		Ceftazidime	
	ESBL positive (n=50)	ESBL negative (n=50)	ESBL positive (n=50)	ESBL negative (n=50)
≤0.25	–	–	–	–
0.5	–	04	–	10
1	–	06	–	08
2	–	34	–	26
4	–	06	–	06
8	–	–	–	–
16	–	–	–	–
32	–	–	14	–
64	06	–	06	–
128	08	–	02	–
256	10	–	10	–
≥512	26	–	18	–

"–": nil.

3.3. Antibiogram of isolated E. coli strains

Under amikacin, for example in Table 4, there are subheadings for N (nosocomial) and C (community) sources; in

Table 4
Percentages of resistance of *E. coli* to aminoglycoside antibiotics.

Quarter	Am		Ge		K		Nt		S		Tb	
	N	C	N	C	N	C	N	C	N	C	N	C
1st	45	35	61	49	36	31	49	39	69	55	61	42
2nd	57	45	68	55	47	42	58	48	71	59	63	47
3 rd	62	52	71	61	51	45	64	54	77	67	74	54
4th	69	61	79	66	54	49	69	59	83	75	78	58
5th	73	64	87	70	56	51	76	66	85	79	79	69
6th	78	66	92	79	59	54	81	71	89	82	81	71
7th	67	65	57	65	76	63	66	71	68	74	79	58
8th	74	79	58	79	85	61	79	75	67	52	61	62
9th	62	71	62	57	64	47	61	64	78	61	68	55
10th	71	68	65	79	76	58	62	76	65	72	78	52
11th	72	78	72	71	63	65	80	81	71	74	81	74
12th	74	81	74	74	78	51	57	68	65	71	79	57
13th	76	79	77	69	73	65	82	77	64	77	70	54
mean±SD	67.7± 9.1	64.9±14.0	71.0±10.8	67.2±9.6	62.9±14.2	52.5±10.0	68.0±10.7	65.3±12.3	73.2±8.3	69.1±9.5	73.2±7.6	57.9±9.2

1st quarter: Nov 2009–Jan 2010; 13th quarter: Nov 2012–Jan 2013. Antibiotic: µg/disc: Am: amikacin 30; Ge: gentamicin 10; K: kanamycin 30; Nt: netilmicin 30; S: streptomycin 10; Tb: tobramycin 10. N: nosocomial; C: community.

Table 5

Percentages of resistance of *E. coli* to β–lactam antibiotics.

Quarter	A		Ak		Au		Az		I		Ox		Pt	
	N	C	N	C	N	C	N	C	N	C	N	C	N	C
1st	27	13	38	22	11	06	15	12	61	33	79	35	34	18
2nd	35	18	41	27	15	11	19	17	63	38	81	47	39	21
3rd	39	22	45	29	18	14	24	19	65	42	85	49	43	26
4th	48	37	49	35	21	17	31	25	67	47	86	53	48	32
5th	61	43	57	39	25	21	39	29	73	52	89	57	53	37
6th	51	45	66	42	31	28	47	32	77	55	92	63	56	46
7th	54	50	62	38	35	23	53	32	76	51	89	52	57	45
8th	59	53	65	37	36	27	58	36	78	56	87	56	66	50
9th	56	52	55	26	43	32	65	40	74	63	84	59	62	53
10th	61	51	57	39	45	36	67	43	77	66	92	54	64	52
11th	62	58	51	44	49	38	62	48	81	64	83	58	65	51
12th	55	61	47	41	51	32	65	49	77	61	89	62	67	58
13th	57	61	56	37	44	35	67	51	76	67	94	69	69	61
mean±SD	51.2±11.0	43.4±16.2	53.0±8.8	35.1±6.8	32.6±13.5	24.6±10.2	47.1±19.3	33.3±12.7	72.7±6.4	53.5±11.0	86.2±4.5	54.6±8.4	55.6±11.5	42.3±14.1

1st quarter: Nov 2009–Jan 2010; 13th quarter: Nov 2012–Jan 2013. Antibiotic: μg/disc: A: ampicillin 10; Ak: amoxyclav 30; Au: augmentin 30; Az: aztreonam 30; I: imipenem 10; Ox: oxacillin 1; Pt: piPERacillin/tazobactam 100/10. N: nosocomial; C: community.

Table 6

Percentages of resistance of *E. coli* to fluoroquinolone antibiotics.

Quarter	Cf		Gf		Le		Na		Nx		Of	
	N	C	N	C	N	C	N	C	N	C	N	C
1st	51	34	84	44	29	13	43	23	82	35	47	23
2nd	53	47	86	49	27	17	49	27	83	43	55	26
3rd	64	50	82	52	35	23	53	32	86	51	59	32
4th	69	53	85	59	36	27	58	36	88	56	67	36
5th	76	58	85	66	43	32	65	40	94	63	74	39
6th	81	61	87	69	45	36	67	43	97	66	72	44
7th	72	68	81	74	49	38	62	48	91	64	73	38
8th	75	61	87	71	51	32	65	49	87	61	69	32
9th	77	51	86	77	34	35	67	51	86	67	74	35
10th	74	59	82	64	43	45	64	50	92	64	73	45
11th	75	64	85	75	47	43	65	49	89	65	67	43
12th	76	69	86	69	51	48	66	48	86	69	71	48
13th	80	67	83	78	55	47	70	52	93	68	75	47
mean± SD	71±9.5	57.1±9.9	84.5±2.0	65.2±11.1	41.9±8.9	33.5±11.1	61.1±8.0	42.2±9.7	88.8±4.4	59.4±10.4	67.4±8.6	37.5±7.8

1st quarter: Nov 2009–Jan 2010; 13th quarter: Nov 2012–Jan 2013. Antibiotic: μg/disc: Cf: ciprofloxacin 5; Gf: gatifloxacin 5; Le: levofloxacin 5; Na: nalidixic acid 30; Nf: norfloxacin 10; Of: ofloxacin 5. N: nosocomial; C: community.

the first quarter, as evident from the samples from community, a total of 48 *E. coli* strains were isolated, of which 35% were resistant to amikacin, 49% were resistant to gentamicin; and so on for other antibiotics presented in other tables (Tables 4 to 7). On the other hand, for the first quarter of study, 33+17=50 strains were isolated from all the 4 nosocomial sources (Table 2), of which 45% of these strains were resistant to amikacin, 61% were resistant to gentamicin and 36% were resistant to kanamycin; and so on and so forth, for the rest of the antibiotics (Tables 4 to 7).

In each progressive quarter, it was seen that percentage of resistance to an antibiotic steadily increased. For amikacin, the resistance percentage of community–acquired pathogenic *E. coli* strains increased from 35% in the first to 45% in the second quarter to 79% in the last (13th) quarter. Similarly in nosocomial section, values increased from 45 to 57% and then to 62% in first

three quarters, and ultimately to 76% in the last/13th quarter (Table 4). A similar situation of progressive increase of percent values of drug–resistant strains from community or wards and cabins or ICU and NICU sources, for each used antibiotics was recorded (Tables 4 to 7).

An examination to percent values of resistance of *E. coli* strains to the aminoglycoside group of antibiotics (Table 4), the β–lactam group (Table 5), fluoroquinolones (Table 6), and the cephalosporin group (Table 7) gives an eyebrow–raising figure for each antibiotic, mostly for *E. coli* strains from nosocomial samples. Perhaps, this high degree of drug resistance should have been due to availability of drugs to patients without any prescription or with prescriptions by quakes or our less–cleanly hospital settings; and patients might be using antibiotics halfway without completing the prescribed course. On detailing, it was found that among the 6 aminoglycosides used against the

Quarter	Ce		Cp		Co–t		Tc	
	N	C	N	N	C	C	N	C
1st	56	30	51	64	51	33	56	29
2nd	64	43	53	69	54	41	64	40
3 rd	72	46	64	72	59	44	72	42
4th	76	49	69	76	65	51	76	46
5th	81	52	76	81	67	54	81	51
6th	85	55	81	85	71	61	85	57
7th	72	51	78	80	66	62	76	72
8th	82	47	84	76	73	68	78	78
9th	75	49	75	72	77	79	87	79
10th	77	51	78	75	80	82	80	82
11th	84	59	81	71	72	75	82	75
12th	81	56	82	82	39	72	79	82
13th	77	58	85	77	31	84	81	84
mean±SD	75.5±8.2	49.7±7.5	73.6±11.2	75.4±5.8	61.9±14.6	62.0±16.5	76.7±8.5	62.8±19.3

1st quarter, Nov 2009–Jan 2010; 13th quarter, Nov 2012–Jan 2013. Antibiotic, µg/disc: Ce, ceftriaxone 30 (cephalosporin); Cp, cefpodoxime 10 (cephalosporin); Co–t, co–trimoxazole 25 (sulfonamide); Tc, tetracycline 30. N, nosocomial; C, community.

E. coli isolates, the maximum resistance patterns were recorded against gentamicin, i.e., 92% in nosocomial isolates and 79% in community isolates. Further, of seven β–lactam antibiotics used, oxacillin was found as the most resistant antibiotic with 94% and 69% resistant values in nosocomial and community isolates, respectively. Moreover, among the 6 fluoroquinolones used, maximum resistant values were recorded against norfloxacin of 97% in nosocomial isolates and 78% for gatifloxacin in community isolates. Similarly, of the 2 cephalosporins, isolates from nosocomial sources had a maximum resistance to ceftriaxone of 85% and in community isolates it was 59%.

4. Discussion

E. coli infections occur in higher magnitude in communities; but in each quarter, the number of resistant *E. coli* strains was increasing in both community and nosocomial sources. Herein, about 40%–60% of strains isolated from urine, pus, swabs both from community and nosocomial sources were with ESBL activity. Imipenem (carbapenem), an active antibiotic against ESBL producing Enterobacteriaceae[24], was found to be degraded to the maximum of 77% by the nosocomial strains, in this study. It is worth to mention here that, this antibiotic was highly stable to β–lactamase activity and had an unusual property of causing post–antibiotic effect on Gram–negative bacteria, on the contrary.

Resistance in *E. coli* to β–lactams was reported first with penicillin and it was due to chromosomally mediated genes[25]; still the β–lactam group of antibiotics are the most common drugs used for the treatment of Gram–negative bacteria and they account for use around 50% for total antibiotic consumptions[26]. Hydrolyzation of newer cephalosporins by ESBLs had been reported to render resistance in them to the third generation cephalosporins namely, ceftazidime and ceftriaxone, as well

as a monobactam, the aztreonam[27]. In this study, it could be confirmed that the ESBL determination by DDST was further confirmed by the determination of MIC values by the micro–titre broth dilution method.

Environmental pollution with resistant pathogenic bacteria had been frequently demonstrated with sewage[28]. Despite high hygienic standards of hospitals, cross contamination of pathogens among patients, and between patients and health personnel should be an important determinant of high nosocomial spread, everywhere. Secondly, drug resistances factors are episome/plasmid mediated and are transferred to other pathogens by the known genetic transfer mechanisms, naturally occurring in sewage drains[28]. Further, device and fomite associated nosocomial infections are directly linked to the dearth in hygiene in hospital environment, due to crowding of patients and their attendants, apart from health personnel. There is no hospital small, airy or well–cleaned, where an enteric pathogen would not get a chance to cause an insidious infection in any immunocompromised patient or a child; the causative MDR bacterium, by the by, should have ample chances to precipitate an episode, by donating resistant genes to other related bacteria in the environment or in a human body culminating in a near universality of the spread of resistance factors among pathogenic bacteria. A healthy individual would have the infection for a period in the cryptic state, while the aged and immunocompromised ones get the infection exposed. The possibility of clones of pathogenic bacteria to be resistant to disinfectants can never be ruled out, as reported for *Pseudomonas*[29].

In India, for community UTI with *E. coli*, norfloxacin, trimethoprim, nitrofurantoin and first generation cephalosporins are usually recommended for the empirical treatment of uncomplicated cystitis, while cephalosporins and aminoglycosides are reserved for parenteral use against complicated infections or pyelonephritis[30]. But many of these

classes of antibiotics were resistant to our isolated strains. In North America particularly, a cut–off point of 20% has been followed as the level of resistance at which, an antibiotic is prevented for further empirical use[31]. Above all, ESBL strains and other strains exhibiting quinolone resistance now threaten the empiric use of both cephalosporin group and ciprofloxacin, seriously impending treatment regimens. Current levels of resistance to antibiotics commonly used locally for empirical treatment, and susceptibility to ampicillin, amoxyclav, cephalexin, cefpodoxime, norfloxacin, amikacin, nitrofurantoin, trimethoprim and imipenem amongst all *E. coli* urinary isolates had been evaluated in India[18]. Tigecycline holds a promise as an alternative choice of therapy for infections caused by ESBL–producing isolates that were carbapenem resistant[32].

Acquisition of a plasmid or transposon bearing a 'resistance gene' confers antibiotic resistance in bacteria[33]. It has been shown in *E. coli* that the multiple antibiotic resistance (*mar*) mutants involve activation of the regulatory *mar*–locus that confers drug resistance by altering the expression of multiple genes[9]. The *mar*–locus had been demonstrated to confer resistance by altering the cell wall, which additionally confers resistance to organic solvents, oxidative stress agents and household disinfectants. This clearly indicated the success of *E. coli* in nosocomial spread. The *mar*–locus was further identified in *Salmonella typhimurium*, and other enteropathogenic bacteria. Expression of a part of *mar*–locus, specified as *mar*A of the emblematic *E. coli* had been reported, to produce the *mar*A phenotype in phylogenetically distant *Mycobacterium smegmatis*, suggesting an over–riding importance of this locus that might have enation with heterolysis in other hosts. Not surprisingly, *Pseudomonas aeruginosa* and *Staphylococcus aureus* both had been reported to acquire the *mar*A locus[34,35]. The *mar* mutants were reported to usually be resistant to low levels of several antibiotics in use. Within clinical chemoprophylaxis tetracycline and rifampicin resistance had been first reported, and it had been shown that resistance was mediated by the plasmid R222, which mutated and conferred easily resistance to fluoroquinolones[36]. It may be now less spectacular of *mar*–mediated antibiotic resistance in other MDR strains of bacteria, but the recognition of the *E. coli mar*–locus is a milestone in identifying regions of bacterial resistance.

Initially 2–3 decades ago, strains with β–lactamase producing genes were controlled by the application of the third generation cephalosporins in 1980's. Later on, a group of plasmid–mediated β–lactamases (known now as ESBLs) capable of hydrolyzing the extended spectrum of cephalosporins, all penicillin derivatives and aztreonam emerged, derived from the old broad spectrum β–lactamases coded genes, TEM–1, TEM–2, (temoneira) and SHV–2 (sulphydryl variable)[37]. Presently, these enzymes are commonly found in *Klebsiella* sp. and *E. coli*. Of course, there are several types of ESBLs, CTX (resistance to cefotaxime), OXA (resistance to ofloxacin), and AmpC, in addition to TEM, SHV derivatives; and the lesser–known types are PER, VEB and BES resistant genes. But, more than 150 TEM types and 30 SHV types of DNA sequences are known to cause remarkable extents of resistance, globally[38]. A typical study from Chandigarh, India recorded that in *E. coli* and *K. pneumoniae*, 30% ESBL positive isolates were with the TEM gene, and an additional 38% isolates were with the SHV gene[39]. It was also demonstrated elsewhere that both TEM–1 and SHV–1 had identical similarities of amino acids in the structural configuration of these two types of β–lactamases[40]. Prevalence of *E. coli* strain harbouring the third most common gene, CTX–M–15 with multidrug resistance has been consistently increasing worldwide[41]. Actually, the worldwide strain of MDR ST131 *E. coli* was reported to possess Incfii plasmids with CTX–M–15 genes; this was the important determinant in community–acquired ESBL *E. coli* isolated from UTI–infections and bacteraemia[42]. Moreover, about in a 13% patients with cancer and neutropenia, *E. coli* associated bacteraemia was often detected, caused by ESBL strains with the CTX–M–15 gene, for which an empiric therapy failed[43]. More recently, NDM–1 (New Delhi metallo–β–lactamase–1) type producing *E. coli* and *K. pneumoniae* strains were reported with CTX–M–15 genes and the associated gene cluster has rendered both these bacteria as superbugs, since those are already classified as PDR bacterial pathogens[6], to put in sotto voce[44]. Clinically, tigecycline was effective against both *E. coli* and *K. pneumoniae*[32], but fosfomycin was effective against ESBL producing *E. coli* only[3].

The emergence of MDR–strains of *E. coli* strains to such a daunting state, lands at gradual loss of self–assurance on antibiotics suggesting a 'post–antibiotic era' ahead, in which the health care system would be at jeopardy. However, we are taking recourse to *Streptomyces* for its prodigious stock of antibiotics. Ironically, when peddled back for three decades or so, the K–12 strain of *E. coli*, known as an inoffensive, rather puny creature lending itself in the revolution of gene–spicing up to industrial/commercial productions of too many human proteins including the insulin for boosting human health, has very competitively related pathogenic strains of same heritage that maraud into enteric and urinary tracts, conflagrating the health domain, with a fear of dizzy pinnacle in future, as the present antibiogram of *E. coli* doggedly vilifies it.

In conclusion, it could be stated that the record of progressive increases of percent values of 1642 isolated *E. coli* strains of drug–resistance to 23 antibiotics in each quarter for each antibiotic elucidates its infection dynamics at an intimidating state with nimble spreads both in hospital and community sectors. The maximum resistance values were recorded against gentamicin (aminoglycoside): 92% and 79% resistance, oxacillin (β–lactam): 94% and 69% resistance, ceftriaxone (cephalosporin): 85% and 59% resistance; and norfloxacin (fluoroquinolone): 97% and 69% resistance, in nosocomial and community isolates, respectively. These were the most frequently used antibiotics for *E. coli*. Without a proper check, MDR *E. coli* strains could cause ominous UTI episodes in slums that have dearth of hygienic milieu.

Soil with spores of such bacteria should infect hospitals at lift floors and nexuses of pathways and corridors. Moreover, adaptation of a uniform, rational antibiotic policy and regular

surveillance are the areas on which, proper and practical care of a hospital should be taken, locally. In addition, to control MDR pathogens, alternate strategies could be further chemical manipulations of drugs in use or search of new complementary/ supplementary drug from other non–microbial source like plants, or the retrospective follow–up to the microbe, *Streptomyces* that has a prodigious stock of unidentified antibiotics.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

Comments

Background

This study was done to record the infection dynamics of pathogenic strains of the most common Gram–negative bacterium, *Escherichia coli* in nosocomial and community sectors by surveillance, over a period of 39 months. Specifically, the prevalence of ESBL producing *E. coli* strains was studied.

Research frontiers

Clinical samples from nosocomial sources, i.e., wards and cabins, ICU and NICU, and community (OPD) sources of the hospital, were used for isolating strains of *E. coli*, which were subjected for testing for production of ESBL enzyme as well as determining antibiotic sensitivity pattern with 23 antibiotics. The ESBL status was further confirmed by determining the MIC of the third generation cephalosporin antibiotics against MDR *E. coli*.

Related reports

Prevalence of ESBL producing *E. coli* and *Klebsiella pneumoniae* isolated from blood stream infections was reported from a hospital of North India.

Ref: Taneja J. *et al*. Nosocomial bloodstream infections from extended spectrum beta lactamase producing *E. coli* and *K. pneumoniae* from GB Pant Hospital, New Delhi. J Infect Dev Countr 2010; 4:517–520.

In a typical surveillance from another developing country of South America, prevalence of ESBL producing bacteria and their molecular characterization was reported.

Ref: Oliveira CF, *et al*. Prevalence of ESBL–producing microorganisms in nosocomial patients and molecular characterization of the SHV type isolates. Brazilian J Microbiol 2010; 41: 278–282.

Innovations & breakthroughs

This study gives systematic information on 1 642 strains of *E. coli*, isolated from different clinical samples and their pedantic antibiogram for 23 antibiotics elucidating the daunting infection– dynamics of MDR *E. coli*, by surveillance in a state in central India. Resistance of *E. coli* to a large number of antibiotics was never reported; a fraction of strains were ESBL producers.

Applications

This work depicts the prevalence of *E. coli* in slums and rural settings as community acquired accounts. This study is anticipated to aid information for designing a specific antibiotics policy for combating drug resistance in *E. coli*. Also it will help the pharmacy world for search for new drugs to control MDR *E. coli* strains.

Peer review

This works gives a good illustration of if infection dynamics of *E. coli* in a typical teaching hospital of developing state of India. Also the study period of 39 months covers a whole cycle of more than 3 years, which substantiates the results obtained in this study. Also the account of ESBL producing *E. coli* strains suggests the rampant spread of drug resistance in hospital and community sectors.

References

[1] Borellio SP, Murray RP, Ferike G. Topley and Wilson's microbiology and microbial infections. In: part V, chapter 52. Ajello, L.;Hay, R. J, editors. *Medical mycology* New York: Wiley; 2010; p.1360–1385.

[2] Song KH, Jeon JH, Park WB, Park SW, Kim HB, et al. Clinical outcomes of spontaneous bacterial peritonitis due to extended–spectrum beta–lactamase–producing *Escherichia coli* and *Klebsiella* species: A retrospective matched case–control study *BMC Infect Dis* 2009; **9**: 41.

[3] Rodríguez–Bano J, Pascual A. Clinical significance of extended–spectrum β–lactamases. *Expert Rev Anti–Infect Ther* 2008; **6**: 671–683.

[4] Klein SJ, Lewinson O. Bacterial ATP–driven transporters of transition metals: physiological roles, mechanisms of action, and roles in bacterial virulence. *Metallomics* 2011; **3**: 1098–1108.

[5] Rho H, Shin B, Lee O, Choi YH, Rho J, Lee J. Antibiotic resistance profile of bacterial isolates from animal farming aquatic environments and meats in a peri–urban community in Daejeon, Korea. *J Environ Monit* 2012; **14**: 1616–1621.

[6] Falagas ME, Karageorgopoulos DE. Pandrug resistance (PDR), extensive drug resistance (XDR), and multidrug resistance (MDR) among Gram–negative bacilli: need for international harmonization in terminology. *Clin Infect Dis* 2008; **46**: 1121–1122.

[7] Anonymous. Diarrhoea: Why children are still dying and what can be done. 2009, ISBN 978–92–806–4462–3 (UNICEF), ISBN 978–92–4–159841–5 (NLM classification: WS 312) (WHO).

[8] Boschi–Pinto C, Velebit L, Shibuya K. Estimating child mortality due to diarrhoea in developing countries. *Bull World Health Organ* 2008; **86**: 657–736.

[9] Sahu MC, Dubey D, Rath S, Debata NK, Padhy RN. Multidrug

Resistance of *Pseudomonas aeruginosa* as known from surveillance of nosocomial and community infections in an Indian teaching hospital. *J Pub Health* 2012; **20**: 413–423.

[10] Pal BB, Khuntiaa HK, Samal SK, Kar SK, Patnaik B. Epidemics of severe cholera caused by El Tor *Vibrio cholerae* O1 Ogawa possessing the CTXB gene of the classical biotype in Orissa, India. *Int J Infect Dis* 2010; **14**: 384–389.

[11] Okonko IO, Nkang AO, Eyarefe OD, Abubakar MJ, Ozejele MO, Amusan TA. Incidences of multidrug resistant (MDR) organisms in some poultry feeds sold in Calabar metropolis, Nigeria. *British J Pharm Toxicol* 2010; **1**: 15–28.

[12] Taneja N, Rao P, Arora J, Dogra A. Occurrence of ESBL & Amp–C β–lactamases & susceptibility to newer antimicrobial agents in complicated UTI. *Indian J Med Res* 2008; **127**: 85–88.

[13] Canton R, Morosini MI. Emergence and spread of antibiotic resistance following exposure to antibiotics. *FEMS Microbiol Revs* 2011; **35**: 977–991.

[14] Ahmetagic A, Pemberton JM. Antibiotic resistant mutants of *Escherichia coli* K–12 show increases in heterologous gene expression. *Plasmid* 2011; **65**: 51–57.

[15] Curova K, Kmetova M, Sabol M, Gombosov L, Lazurova I, Siegfried L. Enterovirulent *E. coli* in inflammatory and non–inflammatory bowel diseases. *Fol Microbiol* 2009; **54**: 81–86.

[16] Fung M, Simpson S, Packer C. Identification of innovation in public health. *J Pub Health* 2011; **33**: 123–130.

[17] El–Mahmood AM, De N, Alo AB. Susceptibility of nosocomial *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* to some antimicrobial drugs routinely used in Adamawa State Hospitals, Nigeria. *J Clin Med Res* 2010; **2**: 125–134.

[18] Bean DC, Krahe D, Wareham DW. Antimicrobial resistance in community and nosocomial *Escherichia coli* urinary tract isolates, London 2005–2006. *Ann Clin Microbiol Antimicrob* 2008; **7**: 13.

[19] Anbumani N, Mallika V. Antimicrobial resistance in community and nosocomial *Escherichia coli* urinary tract isolates from Chennai, South India in 2007. *Intr J Med Up* 2011; **6**: 39–43.

[20] Taneja J, Mishra B, Thakur A, Dogra V, Loomba P. Nosocomial bloodstream infections from extended–spectrum–beta–lactamase–producing *Escherichia coli* and *Klebsiella* pneumonia from GB Pant Hospital, New Delhi. *J Infect Dev Countr* 2010; **4**: 517–520.

[21] Tille P. *Baily and Scott's diagnostics in Microbiology* St. Louis: Mosby, 2002.

[22] Eloff JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Med* 1998; **64**: 711–713.

[23] CLSI – Clinical and Laboratory Standards Institute. Cephalosporin and aztreonam breakpoint revisions fact sheet. Document M100–S20, 2010; Wayne, PA: CLSI.

[24] Ko KS, Lee MY, Song JH, Lee H, Jung DS, Jung SI, et al. Prevalence and characterization of extended spectrum β–lactamase–producing Enterobacteriaceae isolated in Korean hospitals. *Diag Microbiol Infect Dis* 2008; **61**: 453–459.

[25] Drieux l, Brossier f, Sougakoff W, Jarlier V. Phenotypic detection of extended–spectrum β–lactamase production in Enterobacteriaceae: review and bench guide. *Clin Microbiol Infect* 2008; **14**: 90–103.

[26] Falagas ME, Karageorgopoulos DE. Extended–spectrum beta–lactamase–producing organisms. *J Hosp Infec* 2009; **73**: 345–354.

[27] Oliveira CF, Salla A, Lara VA, Rieger A, Horta JA, Alvers SH. Prevalence of extended–spectrum beta–lactamases–producing Microorganisms in nosocomial patients and molecular characterization of the SHV type isolates. *Brazilian J Microbiol* 2010; **41**: 278–282.

[28] Galvin S, Boyle F, Hickey P, Vellinga A, Morris D, Cormica M. Enumeration and characterization of antimicrobial–resistant *Escherichia coli* bacteria in effluent from municipal, hospital, and secondary treatment facility sources. *Appl Environ Microbiol* 2010; **76**: 4772–4779.

[29] Fothergill JL, Winstanley C, James CE. Novel therapeutic strategies to counter *Pseudomonas aeruginosa* infections. *Exp Rev Anti–infective Ther* 2012; **10**: 219–235.

[30] Smet A, Martel A, Persoons D, Dewulf J, Heyndrickx M, et al. Characterization of extended–spectrum beta–lactamases produced by *Escherichia coli* isolated from hospitalized and non–hospitalized patients: emergence of CTX–M–15–producing strains causing urinary tract infections. *Microb Drug Resist* 2010; **16**: 129–34.

[31] Weber DJ, Rutala WA. Central line–associated bloodstream infections: prevention and management. *Infect Dis Clin N Am* 2011; **25**: 77–102.

[32] Kelesidis T, Karageorgopoulos DE, Kelesidis I, Falagas ME. Tigecycline for the treatment of multidrug–resistant Enterobacteriaceae: a systematic review of the evidence from Microbiological and clinical studies. *J Antimicrob Chemother* 2008; **62**: 895–904.

[33] McMurry LM, Levy SB. The Periplasmic protein mppA is not involved in regulation of *mar*A in *Escherichia coli*. *Antimicrob Agents Chemother* 2011; **55**: 4939.

[34] Martínez JL, Rojo F. Metabolic regulation of antibiotic resistance. *FEMS Microbiol Revs* 2011; **35**: 768–789.

[35] Riordan JT, Dupre JM, Cantore–Matyi SA, Singh AK, Song Y, Zama S, et al. Alterations in the transcriptome and antibiotic susceptibility of *Staphylococcus aureus* grown in the presence of diclofenac. *Ann Clin Microbiol Antimicrob* 2011; **10**: 30–40.

[36] Martínez JL. Antibiotics and antibiotic resistance genes in natural environments. *Science* 2008; **321**: 365–367.

[37] Rawat D, Nair D. Extended–spectrum β–lactamases in Gram–negative bacteria. *J Glob Infect Dis* 2010; **2**: 263–274.

[38] Jacoby GA, Munoz–Price LS. The new beta–lactamases. *N Engl J Med* 2005; **352**: 380–391.

[39] Sharma J, Sharma M, Ray P. Detection of TEM & SHV genes in *Escherichia coli* & *Klebsiella pneumoniae* isolates in a tertiary care hospital from India. *Indian J Med Res* 2010; **132**: 332–336.

[40] Majiduddin FK, Palzkill T. An Analysis of why highly similar enzymes evolve differently. *Genetics* 2003; **163**: 457–466.

[41] Peirano G, Costello M, Pitout JD. Molecular characteristics of extended–spectrum beta–lactamase–producing *Escherichia coli* from the Chicago area: high prevalence of ST131 producing CTX–M–15 in community hospitals. *Int J Antimicrob Agent* 2010; **36**: 19–23.

[42] Pitout JD, Gregson DB, Campbell L, Laupland KB. Molecular characteristics of extended–spectrum–beta–lactamase–producing *Escherichia coli* isolates causing bacteremia in the Calgary Health Region from 2000 to 2007: emergence of clone ST131 as a cause of community–acquired infections. *Antimicrob Agent Chemother* 2009; **53**: 2846–2851.

[43] Gudiol C, Calatayud L, Garcia–Vidal C, Lora–Tamayo J, Cisnal M, Duarte R, et al. Bacteraemia due to extended–spectrum–beta–lactamase –producing *Escherichia coli* (ESBL–EC) in cancer patients: clinical features, risk factors, molecular epidemiology and outcome. *J Antimicrob Chemother* 2010; **65**: 3–33–341.

[44] Poirel L, Lagrutta E, Taylor P, Pham J, Nordmann P. Emergence of metallo–beta–lactamase NDM–1–producing multidrug resistant *Escherichia coli* in Australia. *Antimicrob Agents Chemother* 2010; **54**: 4914–4916.