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

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PEER REVIEW

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Comments

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

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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 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.
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[13]. 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 agrandizement of multidrug resistance, from one another[3], eventually landing in 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—heeded 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 pediatric 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).

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 amoxyclillin 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).](image)

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 °C for 18 h. The wells
were examined for the development of pink colour that in a well indi-
cated 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

| 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) | 46(16) | 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, 123 strains were from urine, 104 from swabs, and 46 from pus. Thus, it had been observed

Table 2

| Quarter | Samples from wards and cabins patients | Samples from ICU and NICU patients |
|---------|---------------------------------------|---------------------------------------|
|         | Urine | Swabs | Pus | Body fluids | 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     | 15(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) | 2(–) | 19(10) |
| 6th     | 17(9) | 10(6) | 3(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(–) | 35(15) | 17(9) | 10(6) | 3(1) | 1(1) | 1(–) | 32(17) |
| 10th    | 23(16) | 12(–) | 4(–) | 2(–) | 2(–) | 43(17) | 15(5) | 12(3) | 4(2) | 3(–) | – | 40(19) |
| 11th    | 12(5) | 12(4) | 3(–) | 2(–) | – | 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. OPD, 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 χ²-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 χ²-value was 2.172; the tabulated χ²-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 ≥24 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 |
|-------------------|------------|-------------|
| ≤0.25             | ESBL positive (n=50) | ESBL negative (n=50) |
| 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  | 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 |
| 3rd     | 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 | 63 | 66 | 61 | 61 | 68 | 74 | 79 | 58 |
| 8th     | 74 | 79 | 58 | 79 | 85 | 61 | 79 | 75 | 67 | 67 | 61 | 62 |
| 9th     | 62 | 71 | 62 | 57 | 64 | 47 | 61 | 64 | 78 | 61 | 62 | 55 |
| 10th    | 71 | 68 | 65 | 79 | 76 | 78 | 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 | 57 | 57 | 65 | 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±4.2

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.
the first quarter, as evident from the samples from community, a total of 48 \textit{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 \textit{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 antibiotic was recorded (Tables 4 to 7).

An examination to percent values of resistance of \textit{E. coli} strains to the aminoglycoside group of antibiotics (Table 4), the \(\beta\)-lactam group (Table 5), fluoroquinolones (Table 6), and the cephalosporin group (Table 7) gives an eyebrow-raising figure for each antibiotic, mostly for \textit{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 | A | Ak | Au | Az | I | Ox | Pt |
|---------|---|----|----|----|---|----|----|
| N       | C | N  | C  | N  | C | N  | C  |
| 1st     | 27 | 13 | 38 | 22 | 11 | 06 | 15 |
| 2nd     | 35 | 18 | 41 | 27 | 15 | 11 | 19 |
| 3rd     | 39 | 22 | 45 | 29 | 18 | 14 | 19 |
| 4th     | 48 | 37 | 49 | 35 | 21 | 17 | 31 |
| 5th     | 61 | 43 | 57 | 39 | 25 | 21 | 39 |
| 6th     | 51 | 45 | 66 | 42 | 31 | 28 | 47 |
| 7th     | 54 | 50 | 62 | 38 | 35 | 23 | 53 |
| 8th     | 59 | 53 | 65 | 37 | 36 | 27 | 58 |
| 9th     | 56 | 52 | 55 | 26 | 43 | 32 | 65 |
| 10th    | 61 | 51 | 57 | 39 | 45 | 36 | 67 |
| 11th    | 62 | 58 | 51 | 44 | 49 | 38 | 62 |
| 12th    | 55 | 61 | 47 | 41 | 51 | 32 | 65 |
| 13th    | 57 | 61 | 56 | 37 | 44 | 35 | 67 |

| Quarter | Cl | Gf | Le | Na | Nx | Of |
|---------|----|----|----|----|----|----|
| N       | C  | N  | C  | N  | C  | N  |
| 1st     | 51 | 34 | 84 | 44 | 29 | 13 |
| 2nd     | 53 | 47 | 36 | 27 | 17 | 17 |
| 3rd     | 64 | 50 | 52 | 35 | 23 | 53 |
| 4th     | 69 | 53 | 85 | 59 | 36 | 27 |
| 5th     | 76 | 58 | 85 | 66 | 43 | 32 |
| 6th     | 81 | 61 | 87 | 69 | 45 | 36 |
| 7th     | 72 | 68 | 61 | 59 | 47 | 38 |
| 8th     | 75 | 61 | 67 | 71 | 51 | 32 |
| 9th     | 77 | 51 | 66 | 77 | 34 | 35 |
| 10th    | 74 | 59 | 59 | 64 | 43 | 45 |
| 11th    | 75 | 64 | 58 | 75 | 47 | 43 |
| 12th    | 76 | 67 | 86 | 69 | 51 | 48 |
| 13th    | 80 | 67 | 83 | 78 | 55 | 47 |

Here, the values for the first quarter are given in terms of the number of strains resistant to each antibiotic in each quarter, and the values for the entire study period (13th quarter) are given in terms of the mean percentage of resistance. The abbreviations used are as follows: A: ampicillin; Ak: amoxyclav; Au: augmentin; Az: aztreonam; I: imipenem; Ox: oxacillin; Pt: piperacillin/tazobactam.
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 30% 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, cefazidime 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 marA
of the emblematic E. coli had been reported to produce the
marA 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 marA locus[34,35].
The mar mutants were reported to usually be resistant to
low levels of several antibiotics in use. Within clinical
chemothophylaxis 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
genesis 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 (sulphhydryl 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[44], 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 Streptomycyes 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 death 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, Streptomycetes that has a prodigious stock of unidentified antibiotics.

Conflict of interest statement

We declare that we have no conflict of interest.

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Reports

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 South America, prevalence of ESBL producing bacteria and their molecular characterization was reported.

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 1642 strains of E. coli, isolated from different clinical samples and their pediatric 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 work 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.

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