

# A study on antibiotic resistance pattern of *Escherichia coli* isolated from urine specimens in Eastern India

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## Abstract

**Background:** Antimicrobial resistance is an emerging threat in today's world. Most of the common pathogens are demonstrating significant resistance to commonly used drugs. *Escherichia coli* (*E. coli*) is one of the most common bacterial pathogens in India. There are recent reports of high level of antibiotic resistance in *E. coli*. This will affect the treatment of common conditions such as urinary infection. This is a public health crisis for low- and middle- income countries such as India, as higher antibiotics will cause strain on the already restricted health budget. However, to curb the problem, there is need of clinical data on the prevalence of resistance.

**Objective:** This cross-sectional study was aimed at generating primary data on the prevalence of antibiotic resistance in *E. coli* in a sample population from Eastern India.

**Materials and Methods:** This study was carried out in a tertiary care hospital of Eastern India. Adult patients presenting with urinary tract infection (UTI) were included. Clean catch midstream urine sample was collected and cultured under aerobic conditions. Antibiotic sensitivity was tested by disk diffusion method after liquid culture. Standard descriptive statistical methods were used. Microsoft Excel was used for arranging the data.

**Result:** There were 32 patients in the study with male:female ratio of 10:22. Almost half (47%) of the specimens were resistant to ceftriaxone. Aminoglycoside resistance was found in 25% of the organisms. Fluoroquinolones resistance was also very high with 60% resistant to norfloxacin. *E. coli* specimens of 78% ( $n = 25$ ) were multidrug resistant.

**Conclusion:** Our results give some preliminary data on antibiotic resistance pattern of *E. coli* in this region. This will help in the choice of antibiotics for common conditions such as UTI. However, bigger multicentric studies are needed.

**KEY WORDS:** Antibiotic, urine, *Escherichia coli*, fluoroquinolones, resistance

## Introduction

Antimicrobial resistance is a global health problem with far reaching consequences.<sup>[1]</sup> Antibiotic resistance

among commonly pathogenic bacteria is becoming widespread and this is a threat to effective infection control in near future. This, along with similar drug resistance in virus, parasites, and mycobacteria, threatens to pose a serious challenge to infectious disease management as a whole.<sup>[1]</sup>

There are various causes of increasing antibiotic resistance among bacteria. Indiscriminate use of antibiotics, inappropriate dosing, and incomplete treatment in both humans and also some animals are some of the factors leading to development of resistance in common bacteria.<sup>[2]</sup> Multidrug resistant (MDR) bacterial infections are now becoming quite common, not only in hospital settings but also in the community.<sup>[2]</sup> Thus, this is not just a clinical problem now

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but a public health concern, especially in developing countries where the need for higher antibiotics puts a strain on the already restricted health budget.

*Escherichia coli* (*E. coli*), a common human pathogen of the urinary tract, is well known for being MDR, including resistance to the fluoroquinolones.<sup>[2]</sup> This is especially common in South-East Asia. A very recent study from India showed high level (upto 80%) of expression of drug efflux pumps in *E. coli* specimens, which is indicative of antimicrobial resistance.<sup>[3]</sup> Similarly, another recent study from South India also depicted high levels of resistance to  $\beta$ -lactam and fluoroquinolones antibiotics in *E. coli* specimens isolated from urinary samples.<sup>[4]</sup> Since *E. coli* is the most common uropathogen in India, its drug resistance patterns will have the greatest impact on clinical management and the healthcare costs.<sup>[5]</sup>

However, bacterial antibiotic resistance pattern studies are very rare from Eastern India. Thus, there is a gap in the knowledge base and this makes patient care largely empirical. There are anecdotal reports of increasing drug resistance. This often makes physicians to prescribe multiple antibiotics and also broad spectrum antibiotics, which increase both the cost and the morbidity.<sup>[6]</sup> But in order to curb this problem, some primary data on the resistance patterns is necessary for selecting the appropriate drugs. Unless there is a concrete evidence base, proper selection of antibiotics is not possible.

Thus, we undertook this study in a tertiary care hospital of Eastern India to document the antibiotic resistance pattern of *E. coli* specimens.

## Materials and Methods

We carried out this study in a tertiary care medical college of Eastern India. This was done in the general medicine outpatient departments between 1st October 2014 and 28th February 2015. All patients, male or female, coming with symptoms of urinary tract infection (UTI with dysuria, hematuria, sudden incontinence, or increased frequency of urination) were provisionally included. Then, the nature and purpose of the study were explained in the local dialect and informed consent was obtained. Since this department is only for adult patients, we did not have any paediatric subjects (<18 years) in this study. In subjects with learning disability or cognitive decline, consent of next of kin was taken. Patients with recent (last 3 months) history of catheterization, urinary tract instrumentation, or any perineal surgery were excluded from the study. Any subject with history of any antibiotic use in last 3 months was also excluded. Also any subject who had some physical disability that made self-urine collection difficult was excluded.

The subjects were given sterile wide-mouthed leakproof containers and were asked to collect mid-stream morning urine sample and bring it to the hospital within 2 h. The clean-catch method was explained to them. For subjects with learning difficulty, the carer was entrusted with the job of urine collection. For subjects whose residences were far away

and who, therefore could not travel within 2 h, a spot urine sample was collected in the same way at the outpatient department. The containers were immediately sent to the microbiology laboratory for plating. From the uncentrifuged sample of urine, a preheated and rapidly cooled nichrome wire loop was used to transfer a measure of the sample (10 mL) on to the culture media. The culture media included plain agar and MacConkey agar. Any special media for gram-positive bacteria or fungi were not used. They were then put in an incubator at 37 °C for 24 h. Only specimens showing growth of  $>10^5$  cfu/mL (CFU: colony forming unit), at the end of this period were considered as positive and further tests were carried out on them. Also, growth of more than two types of organisms was considered as a contamination and the result was discarded. However, if there was growth of two organisms and one of them was much more dominant compared to the other, then the dominantly grown organism was taken into account. The growth of bacteria was identified by the colonies of specific colors on the agar plates [Figure 1] and also by suitable biochemical tests. A Gram stain was also carried out for confirmation. However, due to lack of resources, genetic testing was not performed.

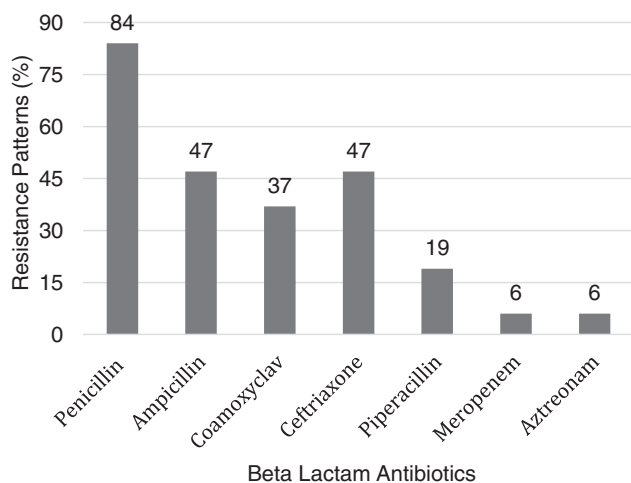
After confirmation of a significant growth, the organism was inoculated into broth for 2 h at 37°C for rapid growth. This allowed the organisms to be in log phase of growth. Then, it was poured on the agar plate containing antibiotic disks. This plate was again incubated at 37°C for 24 h under aerobic conditions. After that time period, the plate was inspected for zone of inhibition around each antibiotic disk [Figure 2]. Each of the zones around the disks was measured to the nearest



**Figure 1:** Culture of organisms after inoculation from urine specimen.



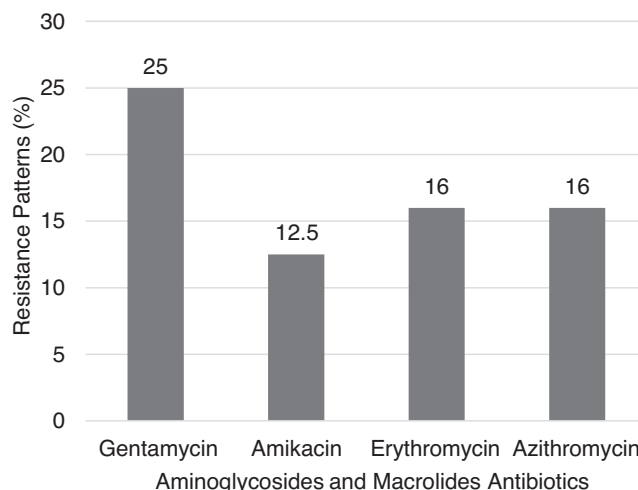
**Figure 2:** Antibiotic sensitivity test by disk diffusion method.



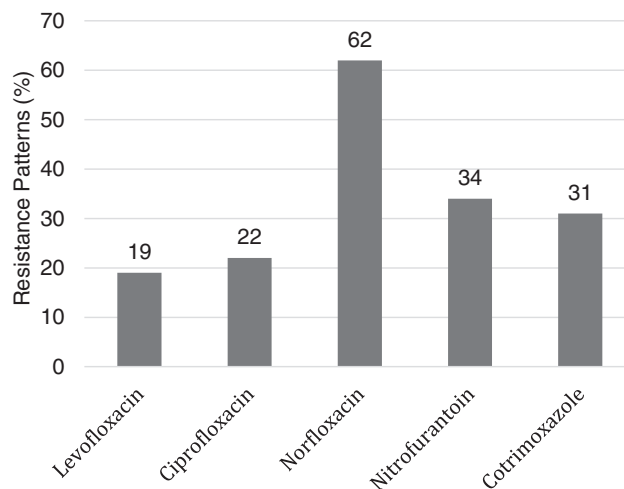
**Figure 3:** Figure showing resistance patterns (in %) for  $\beta$ -lactam group.

millimetre using a standard ruler. Then, using the standard chart provided by the manufacturer of the disks, the zone sizes for each drug were classified as sensitive, intermediate, or resistant. Thus, the final data were ordinal and individual zone sizes were not recorded.

Standard statistical methods were used. The data were arranged in Microsoft Excel worksheet. Descriptive statistical functions were used. Only the specimens showing growth of *E. coli* in the culture were included in the analysis for this study about antibiotic sensitivity.



**Figure 4:** Bar diagram showing resistance patterns (%) for aminoglycosides and macrolides.



**Figure 5:** Bar diagram showing resistance patterns (%) for other antibiotics.

## Result

At first we selected 52 patients for our study. But some patients were excluded according to the criteria stated above and some others showed contaminated specimen. Thus, finally we had 32 patients in the study. The age range of the study subjects was 18–92 years with a mean of 53.2 years. The male:female ratio was 10:22. Most ( $n = 30$ ) of the study subjects were from suburban areas or villages.

The antibiotic resistance pattern is shown separately for different classes of drugs [Figures 3–5]. As seen in Figure 1, 47% of the isolated specimens were resistant to ceftriaxone and almost 20% were resistant to piperacillin. For aminoglycosides [Figure 2], resistance varied from 12.5% for amikacin up to 25% for gentamicin. Macrolide resistance was found in 16%

**Table 1:** Table showing antibiotic resistance patterns (figures indicate % resistant) of urinary pathogens in different Indian studies

Study name, year	Geographical location in India	Organisms	$\beta$ lactam	fluoroquinolones	Nitrofurantoin	Aminoglycosides
Dash et al. (2013) <sup>7</sup>	East	<i>E Coli</i>	58–94	47–53	10	6–16
Shakya et al. (2013) <sup>8</sup>	Central	<i>E Coli</i>	29–41	5–16	nd	nd
Niranjan et al. (2014) <sup>9</sup>	South	<i>E Coli</i>	22–88	75	18	18
Dugal et al. (2013) <sup>10</sup>	West	gram -ve uropathogens	5–80	70–80	15	10–50
This study	East	<i>E Coli</i>	19–84	19–62	34	12–25

nd, no data

cases. Among the other group of drugs, more than 60% were resistant to norfloxacin and more than 30% to nitrofurantoin.

## Discussion

Our cross-sectional study showed that the *E. coli* isolated from urinary specimens were resistant to fluoroquinolones and  $\beta$ -lactam antibiotics in a significant proportion. Similar results have been reported by authors from different parts of India. Table 1 gives a brief comparative overview of the results obtained in different parts of India.

As seen here, *E. coli* isolated from clinical specimens in different parts of India have shown resistance to various antibiotics. The actual antibiotics against whom the organisms are resistant vary from region to region. For example,  $\beta$ -lactam antibiotic resistance seemed to be more common in the East whereas aminoglycoside resistance was higher in the Western part of the country. MDR of *E. coli* is also a concern, not only in India but all over the world. In the study from South India mentioned in introduction, MDR organisms were isolated in 76.5% cases.<sup>[9]</sup> In our study, 78% ( $n = 25$ ) of the isolates were MDR. This is an ominous trend because MDR *E. coli* is a serious threat to infection control.

From earlier studies in India, it was shown that organisms isolated from nosocomial infection cases were more likely to be resistant to multiple antibiotics.<sup>[11]</sup> However, recent (2014) studies have shown that even organisms isolated from community-acquired infections have a very high level of resistance.<sup>[12]</sup> This observation effectively means that even for common infections, the choice of antibiotics becomes restricted. UTI is more common in conditions such as diabetes.<sup>[9]</sup> Thus, antibiotic resistance will have serious augmenting effect on the overall chronic cost of treatment in these diseases.

Different factors have been implicated in increasing antibiotic resistance of bacteria in India. One of the most potent factors is the easy availability of antibiotics over the counter and the tendency of using antibiotics for most symptoms.<sup>[6]</sup> Thus, this is related to access to drugs. Most

of the studies reporting antibiotic resistance in India are from large cities, where people have more access to drugs and thus more chances of misuse exist. Studies from rural areas are rare. One study from a rural teaching hospital in South India showed that out of the isolated *E. coli*, only 1.5% were resistant to nitrofurantoin and only 10% were resistant to amikacin.<sup>[13]</sup> Although this is significant, the resistance data for other antibiotics, including  $\beta$ -lactams were no different from urban figures in the same study. Thus, whether increasing access to antibiotics is the main reason for increasing resistance or whether there is some other inciting factor remains to be discovered.

This increasing trend of antibiotic resistance is a global phenomenon. A study from Thailand showed a high percentage of resistance to common antimicrobials for both *Salmonella* and *E. coli*.<sup>[14]</sup> A study from Iran has also shown the same results for *E. coli*.<sup>[15]</sup> Similar reports have been published all over the world.<sup>[9]</sup> Although there are more studies on *E. coli* due to its ubiquitous nature, studies on other organisms have also revealed a similar trend of antibiotic resistance.<sup>[10]</sup>

This increasing ominous trend demands swift action. Strategies include antibiotic stewardship, publication of protocols, and identification of still useful antibiotics. But these are difficult targets. Even in the United States, it is estimated that up to 50% of antibiotic prescriptions are unnecessary.<sup>[16]</sup> Thus, proper control of antibiotic use, thereby reducing the selection pressure on organisms, is not an easy strategy. In India, studies have also shown very high rate of prescription of antibiotics, both in public and private sectors.<sup>[17]</sup> Sometimes, studies showing antibiotic resistance can act as impetus to prescribe higher antibiotics as physicians may start to think that common drugs will be useless. But a multi-sector coordinated approach is needed to tackle the problem and preserve some medicines for the future.

## Conclusion

Antibiotic resistance is a significant problem with far reaching consequences. Our study shows that the pattern of

antibiotic resistance for *E. coli* in Eastern India is ominous and in some ways, similar to other Indian centers. Further studies on the aetiology of this trend of antibiotic resistance is necessary.

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