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Prospective Study for Antimicrobial Susceptibility of *Escherichia coli* Isolated from Various Clinical Specimens in India

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Abstract

The aim of the present work was to study the prevalence of Extended-spectrum β-lactamases (ESBLs) and Metallo-β-lactamases (Mβ Ls) among 464 *E. coli* clinical isolates obtained from various clinical specimens; and to study the susceptibility of various drugs against *E. coli* isolates. Phenotypic characterization and susceptibility studies were performed according to the methods described in Clinical and Laboratory Standards Institute guidelines (CLSI, 2010). The prevalence of ESBLs and MβLs was analyzed with Polymerase Chain Reaction (PCR), using the previously reported primers.

Among the four hundred sixty four isolates, 186 (40.08%) isolates were ESBLs positive, 75 (16.16%) isolates were MβLs positive, and 80 (17.24%) were both ESBLs and MβLs positive. The remaining 123 (26.50%) were non ESBLs and MβLs. TEM-types ESBLs (bla_{TEM-1}, bla_{TEM-2}, and bla_{TEM-50}) were found in approximately 57% isolates. The prevalence of SHV-types, CTX-M-types and OXA-type was 29.03, 11.82 and 2.15%, respectively. Among the MβLs, the frequency of distribution of NDM-1, IMP-1, VIM-1 and KPC-types was 37.39, 21.33, 18.66, and 22.66%, respectively. In general, 92.6% *E. coli* isolates were susceptible to ceftriaxone plus EDTA plus sulbactam (CSE1034), followed by meropenem (74.4%), imipenem (71.2%), piperacillin plus tazobactam (52.1%), cefoperazone plus sulbactam (46.0%) and amoxycillin plus clavulanic acid (23.6%). Similarly, amoxycillin plus clavulanic acid showed the highest percentage of resistance (72.8%), followed by cefoperazone plus sulbactam (43.6%), piperacillin plus tazobactam (39.3%), imipenem (23.3%), meropenem (20.3%) and ceftriaxone plus EDTA plus sulbactam (CSE1034) (2.5%). Results of the present study revealed that most of the clinical isolates were susceptible to ceftriaxone plus EDTA plus sulbactam (CSE1034), and can be a potent antibacterial agent for the treatment of severe bacterial infections caused by *E. coli*.

Keywords: Antibiotic resistance; *Escherichia coli*; Clinical isolates; Prevalence; CSE1034

Introduction

Escherichia coli is one of the most frequent causes of many bacterial infections, including Urinary Tract Infections (UTI) [1], blood stream infections [2], otitis media [3], pneumonia [4], meningitis [5], traveler's diarrhea, and other infections [6]. Among the antibacterial agents, β-lactam antimicrobial agents are the most widely used to treat bacterial infections, accounting for over 50% of all antibiotics in use [7]. However nowadays, treatment of *E. coli* is becoming increasingly tough because of antibiotic resistance to the agents that are normally prescribed; leading to a therapeutic problem [8]. Wani et al. [9] conducted a susceptibility study in *E. coli* clinical isolates and reported higher percentage of resistance to ceftazidime (99.2%), cefotaxime (99.2%) and ceftriaxone (99.5%). Similarly, Rafay et al. [10] demonstrated 100% resistance of *E. coli* to cephalosporins. Duttaroy and Mehta [11] reported resistance of *E. coli* up to 75% to cefotaxime, 85% to ceftazidime and 60% to ceftriaxone. Kibret and Abera [12] carried out a susceptibility study of erythromycin, amoxicillin and tetracycline on *E. coli* isolated from UTI patients, and found high rates of resistance to erythromycin (89.4%), amoxicillin (86.0%) and tetracycline (72.6%), respectively. Sharma et al. [13] performed a susceptibility study of ceftriaxone, cefotaxime, amoxycillin, cephalexin, ciprofloxacin and gentamicin on *E. coli* and found 80.9, 82.9, 97.9, 89.4, 74.5 and 74.5% resistant, respectively. This high resistance was because of ESBLs production of *E. coli*.

The resistance to the antimicrobials has been increasing over the years and is varying from country to country [14]. Among the causes of β-lactam antibiotic resistance, the production of ESBLs appeared to be most common [7]. The ESBLs are plasmid mediated and can be easily transmitted among members of *Enterobacteriaceae,* thus facilitating the dissemination of resistance, not only to β-lactam, but to other commonly used antibiotics including aminiglycosides and quinolone [15,16]. To overcome ESBLs resistance, carbapenem drugs have been introduced in clinical settings, although carbapenem resistance among the members of the *Enterobacteriaceae* family have been reported increasingly worldwide [17,18]. Resistance in bacteria to carbapenems is due to the production of carbapenem hydrolyzing enzymes called carbapenemases, which is encoded by KPC, VIM and IMP genes [19]. Very recently, Hu et al. [18] demonstrated the lower susceptible of *Enterobacteriaceae* family to imipenem and meropenem, with only 6.5 and 1.3%, respectively.

The increasing rate of the antibiotic resistance and its impact on treatment failure encouraged to study newly reported concept of antibiotic adjuvant entity, by which the increasing failure rate of antibiotics in treatment can be controlled. Information regarding the prevalence of antimicrobial resistance in pathogens can be used for selecting an optional treatment. As far known, there are no recent

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studies regarding antimicrobial resistance among *E. coli* isolates, collected from various specimens in north India zone.

In view of the above data, microbial efficacy of a new Antibiotic Adjuvant Entity (AAE), which is a combination of a non-antibiotic adjuvant Ethylenediamine Tetraacetic Acid disodium (EDTA) along with β-lactam and β-lactamase inhibitor, altogether termed as ceftriaxone plus EDTA plus sulbactam (CSE1034) was studied and compared. The aim of the present study was to study the prevalence of ESBLs and MβLs among *E. coli* clinical isolates obtained from different clinical specimens, and to study the susceptibility of various drugs against *E. coli* isolates.

Materials and Methods

Bacterial isolates and their identification

A total of 464 clinical isolates of *E. coli* were prospectively collected from urine (n=193), blood (n=92), sputum (84), pus (95) from six different centres of north India, including centres from Utter Pradesh, Delhi, Rajasthan, Haryana and Himachal Pradesh. The study was conducted from the period of January 2012 to September 2012. Prior to use, all of the received clinical specimens except blood sample, were inoculated onto Mac-Conkey agar and blood agar, incubated at 37°C for overnight; and the resultant bacterial isolates were sub-cultured and used for further study. With regard to the blood sample, blood was incubated at 37°C overnight in brain heart infusion broth and then a drop of brain heart infusion broth was inoculated on Mac-Conkey agar and blood agar. This bacterial suspension was used as the inoculums, at a concentration of 10^6 colony-forming units (cfu/ml). Re-identification of all *E. coli* isolates was conducted using standard microbiological biochemical tests [20].

Antibiotics

The following antibiotics were used in this study: ceftriaxone plus EDTA plus sulbactam; CSE1034 (30:10:15 µg), piperacillin plus tazobactam (100:10 µg), amoxycillin plus clavulanic acid (20:10 µg), cefoperazone plus sulbactam (75:30 µg), imipenem (10 µg) and meropenem (10 µg). The entire disc was obtained from Hi-Media Laboratories Pvt. Ltd., Mumbai, India.

Antimicrobial susceptibility testing

The antimicrobial susceptibility testing was performed by the disc diffusion method, according to the procedure of Clinical Laboratory Standard Institute guidelines (CLSI, 2010). *E. coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853, *Stenotrophomonas maltophilia* ATCC 13636 were used as the reference strain throughout study.

Screening of isolates for ESBL and MβL production

Screening of *E. coli* isolates for ESBLs and MβLs production was performed according to the procedures as recommended by the CLSI [21], using indicator cephalosporins, ceftriaxone (30 µg), ceftazidime (30 µg) and cefotaxime (30 µg). The respective zone size was interpreted according to the recommendations of CLSI (2010). Isolates exhibiting zone size ≤ 25 with ceftriaxone, ≤ 22 for ceftazidime, and ≤ 27 with cefotaxime were considered possible ESBLs producers [21,22]. Similarly, phenotypic detection of MβLs among the suspected ESBLs producer clinical isolates of *E. coli* was carried out using imipenem (10 µg) and imipenem (10 µg)+EDTA (750 µg) discs, as described earlier [23].

PCR amplification for detection of ESBL and MβL genes

The isolates resistant to at least two cephalosporins were processed for the detection of ESBL genes, TEM-1, TEM-2, TEM-50, SHV-1, SHV-10, CTX-9, CTX-10, CTX-15 and OXA-11, as described previously [24-31]. MβL genes, NDM-1, VIM-1, IMP-1 and KPC-1 were detected, as reported earlier [32-35]. All of the respective primers were obtained from Sigma Aldrich Chemicals Pvt. Ltd., Bangalore, India. For PCR amplifications, about 200 pg of DNA was added to 20 µl mixture containing 0.5 mM of dNTPs, 1.25 µM of each primer and 3.0 µ/unit of Taq polymerase (Bangalore Genei) in 1X PCR buffer. Amplification was performed in a Eppendorf thermal cycler (Germany). The amplified products were separated in 1.5% agarose gel containing 2.5 µl of 10 mg/ml ethidium bromide. The gel was run at 70 volt for 1 h. The gel images were taken under ultraviolet light using gel documentation system (Bio-Rad, USA). A 100 bp ladder (Bangalore Genie) was used to measure the molecular weights of amplified products. The images of ethidium bromide stained DNA bands were visualized using a gel documentation system (Bio-Rad, USA).

DNA isolation

DNA isolation from the clinical isolates was conducted using the alkaline lysis method [36].

Results

Identification and screening of *E. coli*

All of the clinical isolates were found to be *E. coli*. The source of all clinical isolates is shown in figure 1. Out of the 464 clinical isolates of *E. coli*, 186 (40.08%) were observed to be ESBL positive, 75 (16.16%) isolates were MβLs positive, and 80 (17.24%) were both ESBL and MβLs positive. The remaining 123 (26.50%) were regarded as non ESBLs and MβLs.

Diversity of ESBLs and MβLs

Results obtained in the present study showed that TEM-type ESBLs (bla_{TEM-1}, bla_{TEM-2}, bla_{TEM-50}) were found in approximately 57% of the isolates. The prevalence of SHV-type, CTX-M-type and OXA type ESBLs appeared to be 29.03, 11.82 and 2.15%, respectively. Among the MβLs, the frequency of distribution of NDM-1, IMP-1, VIM-1 and KPC-type was 37.39, 21.33, 18.66 and 22.66%, respectively. The detailed distribution of ESBLs+MβLs is illustrated in figure 2.

Antimicrobial susceptibilities of clinical isolates

A result of the *in-vitro* susceptibility testing to various antimicrobial agents in *E. coli* clinical isolates is shown in table 1. A pronounced difference in resistant and susceptibility pattern was observed with the drugs used in the study against the clinical isolates. Susceptibility testing results revealed that approximately 92.6% of *E. coli* isolates were found to be susceptible to ceftriaxone plus EDTA plus sulbactam (CSE1034), followed by meropenem (74.4%), imipenem (71.2%), piperacillin plus tazobactam (52.1%), cefoperazone plus sulbactam (46.0%), and amoxycillin plus clavulanic acid (23.6%). Similarly, amoxycillin plus clavulanic acid showed the highest percentage of resistance (72.8%), followed by cefoperazone plus sulbactam (43.6%), piperacillin plus tazobactam (39.3%), imipenem (23.3), meropenem (20.3%) and ceftriaxone plus EDTA plus sulbactam (CSE1034) (2.5%).

Discussion

E. coli has remained an important cause of infection across the

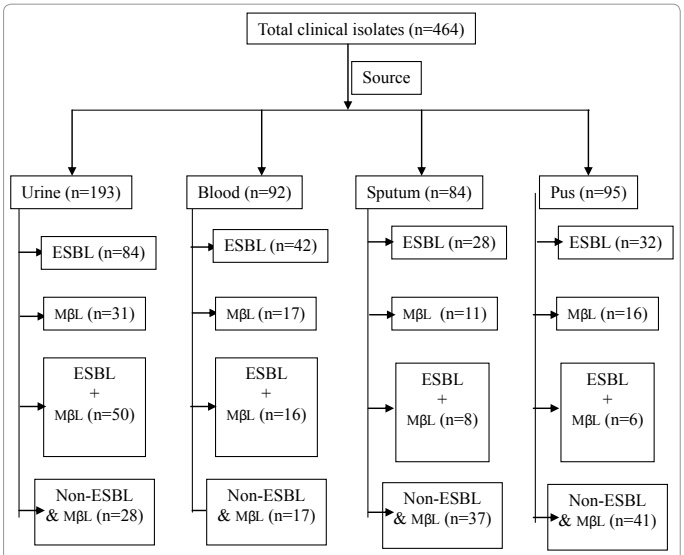

Figure 1: Collection of isolates and their characterization.

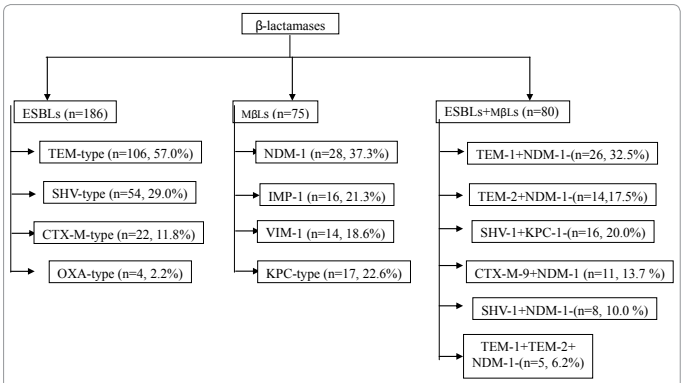

Figure 2: Frequency of β-lactamase genes among *E. coli* clinical isolates.

Antimicrobial agent	Percentage (%) of Isolates		
	Susceptible	Intermediate	Resistant
Ceftriaxone+EDTA+sulbactam	92.6	4.9	2.5
Piperacillin+Tazobactam	52.1	8.6	39.3
Amoxycillin+Clavulanic acid	23.6	3.6	72.8
Cefoperazone+Sulbactam	46.0	10.4	43.6
Imipenem	71.23	5.4	23.4
Meropenem	74.4	5.2	20.3

Table 1: Antibiotic susceptibility of the *E. coli* clinical isolates.

world. Several authors have documented about the antibacterial agents, which are used for the management of *E. coli* infections [8,9]. In this study, a total of 464 clinical isolates of *E. coli,* collected from various clinical specimens were subjected to ESBLs and M*β*Ls screening. Based on the results obtained in the present study, the ESBLs were dominant in blood (45.6%), followed by urine (43.5%), pus (33.7%) and sputum (33.3%). Similarly, the M*β*Ls were dominant in blood (18.5%), followed by pus (17.0%), urine (16.0%) and sputum (13.1%). The collectively ESBLs+M*β*Ls were predominantly present in urine (26.0%), followed by blood (17.4%), sputum (9.5%) and pus (6.3%). Interestingly, non-ESBLs were predominant in sputum (44.0%), followed by pus (43.1%), blood (18.5%) and urine (14.5%). The overall frequency of ESBLs, M*β*Ls and ESBLs+MBLs in *E. coli* isolates was 40, 16.1 and 17.2%, respectively. According to the results obtained in this study, there were increasing trend of ESBLs, ESBLs+M*β*Ls and M*β*Ls in *E. coli* isolates. Previous studies also demonstrated the steadily increasing frequency of ESBLs and M*β*Ls in *E. coli* [9,17,18,37-39].

To the best of the knowledge, there are no studies in north India that have included antibiotic resistance analysis in both ESBLs and M*β*Ls. The results of the antibiotic susceptibility testing performed on the *E. coli* isolates showed that ceftriaxone plus EDTA plus sulbactam (CSE1034) is most active (92.6% susceptibility) against *E. coli* isolates of pus, blood, sputum and urine origin, even when susceptibility to other drugs including carbapenems (meropenem and imipenem) has been lost against MBLs harboring isolates (susceptibility ranged from 23 to 52%). Wattal et al. [40] observed increasing prevalence of carbapenems resistance, varying from 13 to 51% in *E. coli* and *Klebsiella spp*. in New Delhi, India hospitals. Similarly, Gupta et al. [41] also demonstrated high prevalence of resistance, varying from 17 to 22% to various carbapenems among *Enterobacteriaceae* strains.

Surprisingly, ceftriaxone plus EDTA plus sulbactam (CSE1034) was resistant only to those strains which were positive with TEM-50, whereas other comparator drugs were resistant to those isolates, were positive with M*β*L gene including NDM-1, VIM-1, KPC-2, IMP-1, and ESBL genes such as TEM-50. However, ceftriaxone plus EDTA plus sulbactam (CSE1034) appeared to be highly susceptible to isolates harboring MBL positive genes, NDM-1, VIM-1, KPC-2, and IMP-1.

The enhanced susceptibility of ceftriaxone+EDTA+sulbactam (CSE1034) against E. coli is likely to be associated with synergistic activity of ceftriaxone+sulbactam+EDTA. EDTA chelates the divalent ions required for the activity of M*β*Ls, thus enhancing the susceptibility of ceftriaxone plus EDTA plus sulbactam (CSE1034) towards MβLs producing organisms. The EDTA also enhances the susceptibility by altering the outer membrane permeability, which in turn increased penetration of drugs inside the bacterial cells [42].

Results obtained in the current research clearly demonstrate the good *in-vitro* activity of ceftriaxone plus EDTA plus sulbactam (CSE1034) against ESBLs, as well as M*β*Ls producing *E. coli*. However, penems exhibited *in-vitro* activity against only ESBLs producing *E. coli. Hence,* in case of infection with M*β*Ls producing *E. coli*, ceftriaxone plus EDTA plus sulbactam *(*CSE1034) can be of drug of choice for the treatment.

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