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Co-Production of NDM-1 and OXA-48 Carbapenemase, in Urinary Isolates of Escherichia Coli, at a Tertiary Care Centre at Central India

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Abstract
In order to identify genes that encode resistance to carbapenems in urinary isolates of Escherichia coli obtained from patients who were hospitalized at a tertiary care centre in Indore, India. A total of 300 consecutive non-duplicate (one isolate per patient) clinical isolates of Escherichia coli was obtained from urine cultures of hospitalized patients, including those admitted to the medical and surgical intensive care units due to hospital acquired infections, were included in the study. Identification and antibiotic sensitivity assays were performed by standard methods. Polymerase chain reactions (PCR) were utilized to identify the presence of beta-lactamase-encoding genes. All of the isolates exhibited complete resistance to carbapenems and cephalosporins of the second and third generations. In vitro susceptibility of each isolate to tigecycline and colistin was one hundred percent. blaNDM-1 was detected in all of the isolates, and blaOXA-48 co-associated with 55% of the isolates. In conclusion, it was observed that urinary isolates of E. coli co-produced NDM-1 and OXA-48 which were highly resistant to the antibiotics. The timely identification of these genes will contribute to effective infection control and prevention measures by restricting the dissemination of these pathogens.

INTRODUCTION

Antibiotics of the beta-lactam class are frequently used in hospital settings to treat infections caused by Gram-negative bacteria. Escherichia coli, a member of the Enterobacteriaceae family, is frequently encountered and is a significant cause of nosocomial infections. As a result of the presence of extended-spectrum beta-lactamase (ESBL) and Amp C enzymes in these Gram-negative bacilli, carbapenems have emerged as the preferred treatment for these infections in recent times. Multidrug resistance is becoming more prevalent in organisms as a result of gene cassettes encapsulating resistance determinant genes within plasmids, transposons and integrons. The development of carbapenemase, which generates resistance to carbapenems, presents significant obstacles in the management of infections exhibiting pan-resistant phenotypes[1]. In this molecular study resistant genes like NDM-1 and OXA-48 in E. coli were detected.

MATERIALS AND METHODS

The bacterial Clinical Isolates The study was conducted after obtaining due approval from the institutional ethical committee. From Jan 2020-Dec 2023, a total of 300 consecutive non-duplicate (one isolate per patient) clinical isolates of E. coli recovered from urine culture of hospitalized patients admitted to the medical and surgical intensive care units in 1000 bedded tertiary care hospital were included in the study. Collection of urine sample was done using strict aseptic precautions and was immediately processed without any delay. Urine culture was carried out on Cysteine Lactose Electrolyte Deficient (CLED) agar medium using calibrated standard loop. Bacterial identification was performed by routine conventional microbial culture and biochemical tests using standard recommended techniques[2]. Antimicrobial Susceptibility Testing and MIC Determination Antibiotic sensitivity test was performed by standard Kirby Bauer disc diffusion technique as per the guidelines of the Clinical Laboratory Standards Institute (CLSI) with commercially available discs (Hi Media, Mumbai, India) on Mueller Hinton agar plates[3].The antibiotics tested were as follows (potency in µg/disc): piperacillin (100), ticarcillin (75), piperacillin-tazobactam (100/10), ticarcillin clavulanic acid (75/10), ceftazidime (30), cefotaxime (30), cefepime (30),cefoxitin (30), ceftriaxone (30), aztreonam (30), imipenem (10), meropenem (10), ertapenem (10), gentamicin (10), tobramycin (10), amikacin (30), netilmicin (30), ciprofloxacin (5), levofloxacin (5), lomefloxacin (10) and ofloxacin (5). P. aeruginosa ATCC 27853, E.coli ATCC 25922, E. coli ATCC 35218 and K. pneumoniae ATCC 700603 were used as quality control strains. MICs were determined by the E-test (bio Mérieux, France). Phenotypic Screening for the Carbapenemase Production E.coli isolates with reduced susceptibility to meropenem and imipenem (diameter of zones of inhibition =13mm) by disc diffusion method were screened for the production of carbapenemase. The phenotypic detection of the carbapenemase production was performed by the modified Hodge test by using a meropenem disc (10 µg) as per CLSI guidelines[4]. For MHT K. pneumoniae ATCC BAA-1705 and BAA-1706 were used as positive and negative controls, respectively. The screening of metallo-beta-lactamase production was performed by the double-disc synergy tests (DDST) and combined-disc synergy test (CDST) as described previously[5,6]. K. pneumoniae ATCC BAA-2146 and P. aeruginosa ATCC 27853 were used as positive and negative controls, respectively. MBL (IP/IPI) E-test was carried out to detect MBL as per manufacturer's instructions. Molecular detection of the Beta-lactamase genes DNA was extracted using the spin column method (QIAGEN, GmbH, Hilden, Germany) as per manufacturer's instructions. PCR based detection of beta lactamase (ESBL) genes (blaCTXM, blaSHV, blaTEM and blaOXA), Ambler class B MBLs (blaIMP, blaVIM, blaSPM, blaGIM, blaSIM and blaNDM-1),Ambler class D (blaOXA-23, blaOXA-24 and blaOXA48) and for serine carbapenemases (blaKPC, blaGES and blaNMC) were carried out on the isolates by using Gene Amp 9700 PCR System (Applied Biosystems, Singapore)6[7]. PCR products were run on 1.5% agarose gel, stained with ethidium bromide visualized under UV light and photographed.

RESULT AND DISCUSSIONS

Out of total 300 clinical urinary isolates of Escherichia coli, 45 were found to be carbapenem (imipenem, meropenem and ertapenem) resistant by the disk diffusion test and by e-test. These isolates showed resistance to other beta lactam antibiotics, amino glycosides and quinolones tested. Carbapenemase production was confirmed by Modified Hodge test. Production of MBL was confirmed by positive DDST, CDST and MBL (IP/IPI) E-test method. All 45 carbapenem resistant isolates found to be positive for blaNDM-1 and 25 among these isolates found to be positive for blaOXA48.Overall blaCTX-M-15 was the commonest genotype 38/45 (84%) followed by blaTEM32/45(71%), blaSHV28/45(62%) and blaOXA 19/45(42%) either alone or in combination

E. coli is a common cause of community-acquired and health-care-acquired infections. Carbapenems are being increasingly used to treat infections due to multi drug resistant Enterobacteriaceae and sometimes empirically. This has got a major impact in the emergence of multi drug resistance which can be easily transmitted from one species to another by transferable elements such as plasmids. MIC values for

imipenem, meropenem and ertapenem ranged from 8-64 µg/ml. Strains found to harbor both blaNDM-1 and blaOXA-48 showed higher MICs against carbapenems (64 µg/ml) as compared to MICs (8-16 µg/ml) showed by strains harbouring blaNDM-1 only. Isolates were found to be susceptible to tigecycline and colistin as per MIC breakpoints. In this study, 45 (100%) blaNDM-1 positive E. coli isolates showed positive results from the modified Hodge test while finding from Castanheira M *et al.,* reported the occurrence of weakly positive results for the modified Hodge test in the detection of NDM-1 producing Enterobacteriaceae[8]. There was a 100% correlation with positive DDST, CDST and MBL (IP/IPI) E-test method with the presence of NDM-1in these clinical isolates as detected by PCR. The overall co-presence of blaOXA-48 and blaNDM-1 among E. coli in our study was found to be (25/300) 8.3%. Among ESBL blaCTX-M-15 was the commonest genotype 38/45 (84%) followed by blaTEM 32/45(71%) blaSHV 28/45 (62%) and blaOXA 19/45(42%) either alone or in combination in the blaNDM-1 producing E.coli. Previous studies from India had reported the presence of TEM-1, CTX-M-15, SHV-1, SHV-12, DHA and CMY-2 and in the NDM-1 producing Enterobacteriaceae[9,10] .While findings from other studies from abroad had showed the presence of blaCTX-M-15, blaTEM-1, blaSHV-28, blaSHV-11, and blaCMY-6 in the blaNDM-1 possessing Enterobacteriaceae[11,12]. Though, the strain remains sensitive for tigecycline in vitro but it is not recommended for use in UTI infections. Colistin is the main stay of therapy.

CONCLUSION

Both blaNDM-1 and blaOXA-48 resulted in higher MICs against carbapenems (64 µg/ml) than presence of blaNDM-1 alone (>8-32µg/ml). This must be extremely worrisome, as dissemination of plasmids carrying resistant determinant genes from one species to another makes organism refractory to the common antibiotics used in clinical practice. Here we report the co-presence of NDM-1 with OXA-48 producing E.coli in urine culture from a tertiary care centre in central India. Early detection of these resistant determinant genes by molecular methods is essential in limiting the spread of infection due to these organisms.

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