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A study on Carbapenamase resistance UTI causing *Klebsiella pneumoniae* godavari districts

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ABSTRACT

Antimicrobial resistance (AMR) is the burning issue in the medical science. Enterobacteriaceae that are resistant to carbapenems considered as carbapenem resistant (CR). Hence a study was conducted to find phenotypic as well as genotypic CR Klebsiella pneumoniae (KP). It was a prospective study, conducted in the department of Microbiology, Vishnu dental college, Bhimavaram. Informed written consent was taken from all the study participants. Individuals aged ≥ 18, with the symptoms of UTI of both gender were included in this research. Midstream urine (MSU) samples were collected and labelled properly, specimen were transported immediately to the lab cultured as per the guidelines. All KP isolates were processed for antimicrobial susceptibility test. Simultaneously, Nucleic acid (NA) extraction was carried by using kit method, PCR was carried to detect CR genes, namely blaVIM-1, blaNDM-1, blaIMP. The association between two variables was done by Chi-Square test, P > 0.05 was considered to be statistically significant. Urinary tract Infection was detected in 322, highest prevalence in 29 – 37 years. Out of the 52 CR KP isolates, OXA 48 was detected maximum (31). Gender wise, 10 were male and 24 female participants; age was ranged between 29 to 47 and the mean age was years. Regular survey on hospital acquired infections (HAIs) with timely revision of antibiotic policy and thorough implementation may reduce the occurrence CR.

Keywords: *Antibiotic, urine, infection*

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INTRODUCTION

Antimicrobial resistance (AMR) is the burning issue in the medical science. Globally, AMR nuisance is significant and 700,000 deaths were reported, annually [1]. Indiscriminate as well as increased usage of antibiotics in food as well as agricultural sectors are the leading causes not only for the AMR and also for the spread of genes responsible for this drug resistance (DR) [2]. At this point, extended spectrum betalactamase (ESBL) is a complex, plasmid mediated and rapid spreader. Carbapenems and antibiotics having ESBL inhibitor are the only exceptions for ESBLs [3].

Worldwide, Urinary tract infection (UTI) is the commonest bacterial infections. UTI also require significant hospital care as this may leads to urosepsis and sepsis [4, 5]. Gram negative rods (GNR) are common causative agents of UTI, Enterobacteriaceae are the major contributors [6, 7]. However, there is raise in UTI caused by non-lactose fermenting bacteria [8].

β lactam antibiotics are the common antimicrobial agents used to treat infections caused by GNR. Especially in the ICUs, there is increased utility of carbapenems, next to extended spectrum penicillins as well as cephalosporins [9]. Carbapenem resistance (CR) is a popular clinical problem; GNR with CR is reported throughout the globe [10]. CR bacteria that cause UTI is top infection [8].

Enterobacteriaceae that are resistant to one or all of the carbapenems such as ertapenem, meropenem, imipenem or doripenem is considered to be CR [11]; there is increase in carbapenem resistant enterobacteriaceae (CRE) [12]. CRE leads to high mortality as well as serious public health threat [13]. Due to raise in CRE, early detection is the essential. Hence a study was conducted to find phenotypic as well as genotypic CR *Klebsiella pneumoniae* (KP).

MATERIAL AND METHODS

It was a prospective study**,** conducted in the department of Microbiology, Vishnu dental college, Bhimavaram. Study was conducted for a period of 12 months. Study protocol was approved by the institutional Ethics committee. Informed written consent was taken from all the study participants.

Individuals aged ≥ 18, with the symptoms of UTI of both gender were included in this research. Individuals < 18 years, those on steroid treatment, malignancy, transplant recipients and noncooperative individuals were excluded from the study.

Sample size was calculated using formulae $n = 4pq/l^2$. Here prevalence (p) will be taken at 40% as per the S.Bobbadi et al. report. Q = 100 – P; 100 – 40 = 60. Error was taken at the rate of 10% of P. with these the sample size was considered to be 600 [14].

After recruiting the patients in the study, detailed clinical history was collected. All the findings were recorded in the study proforma. The study was clearly explained in the local language. The participants were allowed to ask doubts. After clarifying all the doubts, midstream urine (MSU) sample collection was explained in the local language. Once the participant is comfortable, then MSU was collected and labelled properly [15]. After successful collection and labelling, all the specimen were transported immediately in self-sealing polythene covers with two compartments; the laboratory requisition form is placed in one and the sample in the other compartment. Then the samples were transported to the laboratory immediately [16]. If there is any delay, for a period of > 1 – 2 hrs, samples were refrigerated.

Pyuria was considered to be the important criteria for the diagnosis of UTI [17]. Hence, immediately after receiving the sample in the laboratory, urine wet mount was conducted to find the pus cells, pyuria. In this study, ≥ 8 pus cells were considered to be significant. Simultaneously, samples were inoculated by semiquantitative method on Cysteine lactose electrolyte deficient (CLED) agar along with MacConkey agar (MA) and blood agar (BA). After inoculation, plates were incubated at 37^0C for 48 hrs. After 24 hrs incubation, plates were observed for growth. For the identification of the isolates, Gram stain (GS), hanging drop examination, biochemical reactions were conducted and antimicrobial susceptibility test was also performed to find the drug susceptibility. Antimicrobial susceptibility test will be done by the conventional modified Kirby-Bauer disc diffusion method on Mueller-Hinton agar medium. Commercially available HIMEDIA antibiotic discs containing a known concentration of antimicrobial agent will be used in the test. CLSI recommendations were followed [18].

Nucleic acid (NA) extraction was carried by using kit method as per the manufacture guidelines. Finally elute was stored at – 20^0C for further PCR reaction. PCR was carried to detect CR genes, namely *bla*VIM-1, *bla*NDM-1, *bla*IMP [19, 20]. PCR amplification was performed using 20 µl of total reaction volume under the following conditions: one cycle at 95^0C for 15 min, then 40 repeated cycles of 15 seconds denaturation at 95^0C for, 30 seconds annealing at 55^0C with *bla*OXA-48 or 52^0C with *bla*VIM-1 and *bla*NDM-1 and 1 min extension at 72^0C [21].

Statistical analysis

Statistical analysis will be conducted using SPSS version 21. Data were analyzed by Mean ± SD for continuous variables and percentage for categorical data. The association between two variables was done by Chi-Square test for categorical data, P values less than 0.05 was considered to be statistically significant.

RESULTS

Total 610 MSU samples were collected in this research; in this UTI was detected in 322 (100%). The prevalence of UTI was identified to be highest in 29 – 37 years and the mean age was 37.2 years. The female male ratio was 1.7. Out of the 313 (100%) bacterial isolates, Esch.coli was the most prevalent (29%; 91) followed by KP (25%; 78).

Out of 78 KP isolates, 100% (78/78) were resistant to ampicillin, followed by Nitrofurantoin (82%; 64), Norfloxacin (78%; 61), Ceftriaxone (78%; 61), Imipenem (74%; 58), Cefixime (72%; 56), Meropenem (79%; 52), Amikacin (60%; 47) (Table 1).

Out of the 52 (100) carbapenem resistant KP isolates, OXA 48 was detected in maximum (31; %) followed by VIM-1 (4), *bla*NDM-1 (3), *bla*IMP (2). In this, Gender wise, 10 were male and 24 female participants; age was ranged between 29 to 47 and the mean age was years.

Table 1: Sensitivity pattern of different antibiotics used in the study.				
Mode of action	**Antimicrobial category**	**Antibiotic**	**Sensitive**	**Resistant**
Cell wall synthesis inhibitors	Aminopenicillin	Ampicillin (10 µg)	0	78 (100)
	ESBL	Ceftriaxone (30 µg)	17 (22)	61 (78)
		Cefixime (5 µg)	22 (28)	56 (72)
	Carbapenems	Imipenem (10 µg)	20 (26)	58 (74)
		Meropenem (10 µg)	16 (21)	52 (79)
Protein synthesis inhibitors	Aminoglycosides	Amikacin (30 µg)	31 (40)	47 (60)
Nucleic acid synthesis inhibitor	Quinolones	Nalidixic acid (30 µg)	39 (50)	39 (50)
	Fluoroquinolones	Ciprofloxacin (5 µg)	17 (22)	61 (78)
		Norfloxacin (10 µg)	15 (20)	63 (80)
	Nitrofurans	Nitrofurantoin (300 µg)	14 (18)	64 (82)
Folate pathway inhibitors	Trimethoprim and sulfamethoxazole	Cotrimoxazole (25 µg)	31 (40)	47 (60)

DISCUSSION

UTIs are the serious and frequent bacterial infections; responsible for 7% of febrile illnesses [22]. There is delay in the diagnosis as well as treatment of UTI due to nonspecific symptoms. Urine culture is the standard technique for the diagnosis of UTI [23].

In this research, just 8% urine samples showed significant bacteriuria (SB) ($\geq 10^5$ CFU/ml). It was reported to be around 17% [24] and also reported to be >20% [25]. There is no correlation in the numerical values of SB. In this research we included adults with signs and symptoms of UTI. Most of the study participants are on antimicrobial treatment. Hence there was less incidence of SB. But when the patient was on antibiotic treatment, even a single colony is also significant [26].

Pyuria can be used as an important marker in the diagnosis of UTI [27]. Hence microscopic pyuria is considered to be the presumptive evidence for the diagnosis of UTI by the clinicians. In this research, individuals with signs and symptoms of UTI were recruited. Hence pyuria was diagnosed in all; similar findings were reported in the literature also. But urine culture was only considered for the diagnosis of UTI in this study because pyuria may present in absence of UTI [28].

The mean age of participants with UTI in this study was 37.2 years; usually this is sexually active age group. Definitely there is exchange of flora during the sexual intercourse on either side. This could be the reason for high incidence of UTI in the sexually active group. In this research, the female male ratio for UTI was 1.7. Generally, due to the short urethra, more UTI chances among the women. Hence high prevalence was identified in female.

In this research most of the pathogenic bacterial isolates were GNRs. The previous reports also reveals similar finding [25, 29]. Out of the 313 (100%) bacterial isolates, Esch.coli was the most prevalent (29%; 91) followed by KP (25%; 78) (Table 1). In the literature also, Esch.coli was reported to be the leading UTI causing agent because it is one of the floral members in the urethral region as well as gastro intestinal tract. So that can get exchanged during sexual intercourse and ascend urethra frequently than other uropathogens [30]. Adding to this, the virulence properties of E. coli such as P-fimbriae, hemolysins, binding ability to the uroepthelium contribute E. coli to cause UTIs [31]. So that this could be the common agent that cause UTI.

Due to the release of enzyme carbapenamases, bacteria are resistant to β-lactam group antibiotics. [32] This also leads resistant to other antibiotics due to the expression of multiple drug resistant genes. [33] Finally ends to multiple drug resistance [34]. In this research 52 KP strains were resistant to carbapenems. In the reported literature, KP is the leading cause of CR [24]. Release of carbapenemase, increased expression of efflux pump and change in pencillin binding proteins are the major contributor factors of CR especially in GNR [35]. In this study, OXA 48 gene was detected in maximum (31; %) in CR KP; followed by VIM-1 (4), blaNDM-1 (3), blaIMP (2). Similar findings were reported in other global studies. [36] Not only in KP, OXA 48 is the leading CR in Esch.coli also. Hydrolytic activity on carbapenems and cephalosporins is another mechanism of OXA 48 [37].

DR causing microbes can spread easily from person to person; improper management of infections, in appropriate usage of the antimicrobials, food habits and so on are responsible for this spreading. [38] In this area usually antibiotics are purchased in the pharmacy without prescription. Ones the patient is little

relieved from the symptoms, have a habit of discontinuing the usage and nobody follow national antibiotic policy. Usually people visit non-qualified practitioners. Just to attract them as business strategy they prescribe wide spread antibiotics to treat common infections. Moreover usually most of these prescriptions are issued without culture and antibiotic sensitivity testing.

CONCLUSION

Regular survey on hospital acquired infections (HAIs) with timely revision antibiotic policy as well as thorough implementation of antibiotic policy may reduce the occurrence DR. Further, there should be regular infection control training to control the HAI. In addition to this the health care workers should be trained thoroughly, to adopt the infection control protocol and to implement the infection control program. We can't detect the phenotypic DR using recent techniques and single center research are the limitations.

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