JMID/
Journal of Microbiology and Infectious Diseases

2018; 8 (4):153-157
doi: 10.5799/jmid.493854

RESEARCH ARTICLE

Antibiotic Therapy for ESBL and NDM producing *Escherichia coli* and *Klebsiella pneumoniae* isolates in a Tertiary Care Center

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ABSTRACT

Objective: Antibiotic resistance is a global phenomenon wherein physicians face the most challenging decision to make for the right kind of drug, and its combinations, to tackle the ever growing ESBL and NDM on pathogens. The aim of the study is to understand outcome of hospitalized patients undergoing antibiotic therapy for bacterial infections. These patients are from Kamrup District of Assam, India.

Materials and methods: A total of 185 clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* were collected from hospitalized patients of a tertiary care centre presenting symptoms of infection. Upon biochemical identification, their antibiotic susceptibility were assessed; isolates resistant to 3rd or 4th generation cephalosporins or carbapenem were phenotypically and genotypically determined for the production of extended spectrum β-lactamase (ESBL) and carbapenemase. PCR was carried out for CTX-M, TEM, SHV, OXA-48 and New Delhi Metallo-β-lactamase (NDM).

Results: Bacterial infection is ubiquitous among male and female patients, with high isolation rate of *Escherichia coli* among female patients with urinary infection. The highest resistance against *E. coli* isolates was nalidixic acid (82.9%; p≤0.005) and cefixime (81.4%; p≤0.005). The highest resistance against *K. pneumoniae* was cefotaxime (77.7%; p≤0.005) and ceftazidime (73%; p≤0.005). Imipenem was the most effective antibiotic while ertapenem was the least. Antibiotic therapy included piperacillin (alone or in combination with tazobactam) for both *E. coli* and *K. pneumoniae* infections.

Conclusions: Ceftriaxone, amikacin, cefepime, ceftriazone and imipenem were chosen as treatment options; isolates showed intermediate to negligible resistance to these drugs. Tigecycline was administered to patients infected with NDM producing pathogens. *J Microbiol Infect Dis 2018; 8(4): 153-157.*

Keywords: *antibacterial agents, drug resistance, ESBL, NDM*

INTRODUCTION

β-lactamase mediated antibiotic resistance is a worldwide health threat [1,2]. The choice of drugs administered to infected patients is primarily based on the knowledge of the antibiotic resistance patterns within the given geographical boundary which is crucial for the initial empirical antibiotic therapy [3]. The antibiotics that are clinically appropriate for any given bacterial infection are ineffective in inhibiting bacterial infection. This result in antibiotic discordance wherein the causative organism demonstrates in vitro non susceptibility to the empiric antibiotic therapy administered. The aim of this article is to report the antibiotic resistance trend in hospitalized patients undergoing antibiotic therapy. The prevalence of β-lactam antibiotic resistance is correlated with patient demographics and status for those presenting *Escherichia coli* and *Klebsiella pneumoniae* infection in a tertiary care hospital.

METHODS

Sample design

The clearance certificate was procured from the Institutional Ethical Committee of Gauhati University (GU/ACA/Ethics/2012/3993 dated 10/1/12). A total of 185 non-repetitive *E. coli* and *K. pneumoniae* isolates were collected from hospitalized patients presenting symptoms of

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Received: 16 February 2018 Accepted: 30 August 2018

bacterial infection. These isolates were then identified by Gram's staining and biochemical tests (IMViC tests) [4]. The specimens collected include urine (n=83), sputum (n=53), pus (n=20), blood (n=11) and others (n=18) comprising of wound swab, high vaginal swab, and throat swab. Patients' information in terms of demographics, age, gender, disease condition was collected from the respective departments.

Antibiotic susceptibility test and β-lactamase production

The antibiotic susceptibility testing was carried out for commonly used antibiotics – 3rd and 4th generation cephalosporins, nalidixic acid and carbapenems as per the guidelines by the Clinical and Laboratory Standards Institute, 2011 [5]. Isolates resistant to any 3rd or 4th generation cephalosporin (cefexime, ceftazidime, cefotoxime, cefexime), and any carbapenem (imipenem, ertapenem, doripenem, meropenem) were selected for further analysis. Phenotypic characterization for the production of extended spectrum-β-lactamase (ESBL) and carbapenemases was carried out by Double Disk Synergy Test (DDST) [5] and presence of metallo-β-lactamases by Double Disk Diffusion test employing Ertapenem (5 ug) and Ertapenem + 0.5M EDTA [6].

Molecular detection of β-lactamase genes

PCR confirmation was performed for ESBL and carbapenemase genes including Ambler class A (blaCTX-M, blaTEM, blaSHV and blaKPC), class B (blaVIM, blaIMP, and blaNDM), and class D (blaOXA-48). Primers for molecular detection were taken from published literature [1, 7-9]. Positive controls used for the multiplex PCR were *K. pneumoniae* ATCC 700603 (SHV-18), J53 pMG298 (CTX-M-15) and autoclaved distilled water was used as negative control. PCR positive TEM, OXA-48 and NDM amplicons were sequenced and compared to the Genbank database for confirmation of correct amplification of target gene; these isolates were then used as positive controls.

RESULTS

Clinical samples were collected from hospitalized patients who are from Kamrup district of Assam. These patients are admitted to various departments namely male medicine unit, female medicine unit, intensive care unit, urology and nephrology department, post-operative care ward, gynecology (septic) ward, paediatric ward, etc. Patients with urinary tract infections, respiratory infection, catheter-based infections, and septicemia in cancer patients, patients with diabetes, ear nose throat infections, pot operative infection, and infection in pregnancy constitute the sampling pool in this study. Of which patient with urinary infections and chronic kidney disease (44.3%) were higher in number followed by infections of the respiratory tract (20.9%) and during pregnancy (15.8%). Further classification of the diagnosis along with patient age and gender is presented in Table- 1, along with the distribution of *E. coli* and *K. pneumoniae* isolates. Of a total of 185 clinical bacterial isolates collected, *E. coli* isolates showed the highest antibiotic resistance to nalidixic acid (30 µg) (87/105, 82.9%; p≤0.005) followed by cefixime (5 ug), (85/105, 81.4%; p≤0.005), while the most effective antibiotic (least resistance shown) was imipenem (10 µg), (9/105, 8.4%; p≤0.005) and nitrofurantoin (30 µg) (33/105, 31.2%; p≤0.005). Among *K. pneumoniae*, highest resistance was documented against cefotaxime (30 µg) (62/80, 77.6%; p≤0.005) and ceftazidime (10 µg) (58/80, 73.1%; p≤0.005) while the most effective antibiotic (least resistance shown) was imipenem (10 µg) (9/80, 11.2%; p≤0.005) and chloramphenicol (30 ug) (12/80, 15.4%; p≤0.005). Ertapenem is one antibiotic that showed a high resistance in both groups of bacterial isolates: 93/105 (88.9%; p≤0.005) and 63/80 (84.2%; p≤0.005), respectively. Amongst ESBL producing *E. coli*, PCR positive results for CTX-M, TEM and SHV was observed for 43, 37 and 12 isolates, whereas ESBL producing *K. pneumoniae* showed positive PCR results in 32, 32 and 37 isolates, respectively. Furthermore, it was observed that the percentage of CTX-M (41% vs 39%; p≤0.005) and NDM (21.9% vs 12.2%; p≤0.005) genes detected was significantly higher among *E. coli* compared to *K. pneumoniae*, respectively and TEM (39.1% vs 35.2%; p≤0.05) and SHV (45.1% vs 11.4%; p≤0.005) genes were detected higher among *K. pneumoniae* than in *E. coli*, respectively. PCR detection of OXA-48 was reported for the first time in Assam [10] and upon further screening among all samples reported here, there were 5 isolates carrying this β-lactamase gene with a

Table 1. Characteristics of patients with *E. coli* and *K. pneumoniae* infection

Characteristics	*Escherichia coli*					*Klebsiella pneumoniae*				
	Non β-lactamase producer (n=45) n (%)	**ESBL producer (n=57)** n (%)	**NDM (n=23)** n (%)	**OXA-48 (n=2)** n (%)	**Total (n=105)** n (%)	**Non β-lactamase producer (n=35)** n (%)	**ESBL producer (n=45)** n (%)	**NDM gene producer (n=12)** n (%)	**OXA-48 (n=3)** n (%)	**Total (n=80)** n (%)
Age										
≤18 years	5 (11.1)	11 (19.3)	5 (21.7)	1 (50)	16 (16.2)	4 (11.4)	5 (10.9)	1 (8.3)	ND	10 (12.2)
19-45 years	22 (48.8)	27 (47.4)	8 (34.8)	ND	49 (46.7)	16 (45.8)	21 (45.6)	6 (50)	1 (33.4)	37 (45.1)
≥46 years	18 (40)	19 (33.3)	10 (43.5)	1 (50)	37 (36.2)	15 (42.8)	20 (43.3)	5 (41.7)	2 (66.6)	35 (42.7)
Sex										
Male	16 (35.5)	26 (45.6)	11 (47.8)	1 (50)	42 (40.9)	27 (77.14)	31 (67.4)	7 (58.3)	2 (66.6)	59 (72)
Female	29 (64.5)	31 (54.4)	12 (52.2)	1 (50)	60 (59.1)	8 (22.85)	15 (32.6)	5 (41.7)	1 (33.4)	23 (28)
Diagnosis										
Urinary Tract infections	7 (15.5)	14 (24.6)	5 (21.7)	1 (50)	21 (20)	1 (2.85)	5 (11.1)	1 (8.3)	2 (66.6)	6 (7.5)
Chronic Kidney Disease	5 (11.1)	10 (17.5)	7 (30.4)	ND	15 (14.3)	2 (5.71)	ND	ND	ND	2 (2.5)
Chronic Liver Disease	2 (4.4)	3 (6.6)	2 (8.6)	ND	5 (4.8)	ND	4 (8.8)	1 (8.3)	ND	5 (6.2)
Tuberculosis	4 (8.8)	ND	ND	ND	4 (3.8)	2 (5.71)	ND	ND	ND	2 (2.5)
Enteric fever	1 (2.2)	2 (3.5)	ND	ND	3 (2.8)	ND	1 (2.2)	ND	ND	1 (1.3)
Respiratory Infections	1 (2.2)	ND	ND	ND	1 (0.9)	7 (20)	9 (20)	2	ND	16 (20)
Pregnancy	4 (8.8)	6 (10.5)	3 (1.3)	1 (50)	10 (9.5)	2 (5.71)	3 (6.6)	1 (8.3)	ND	5 (6.2)
Tonsilitis	ND	ND	ND	ND	ND	3 (8.57)	2 (4.4)	ND	ND	5 (6.2)
Chronic Obstructive Pulmonary Disease	ND	ND	ND	ND	ND	2 (5.71)	2 (4.4)	1 (8.3)	ND	4 (5)
Pnuemonia	ND	ND	ND	ND	ND	2 (5.71)	1 (2.2)	ND	ND	3 (3.8)
Fracture/Bone injury	1 (2.2)	1(1.7)	ND	ND	2 (1.9)	2 (5.71)	1 (2.2)	1 (8.3)	ND	3 (3.8)
Brain injury	2 (4.4)	1(1.7)	ND	ND	3 (2.8)	2 (5.71)	4 (8.8)	ND	ND	6 (7.5)
HIV positive	ND	ND	ND	ND	ND	ND	2 (4.4)	1 (8.3)	ND	2 (2.5)
Blood Infections	4 (8.8)	ND	ND	ND	4 (3.8)	ND	ND	ND	1 (33.4)	ND
Other diseases	10 (2.2)	20 (35.1)	10 (43.5)	ND	32 (30.5)	11 (31.42)	9 (20)	1 (8.3)	ND	20 (25)

ND: Not Detected
ESBL: Extended Spectrum β-lactamase
NDM: New Delhi Metallo-β-lactamase

total prevalence percentage of 1.9% and 3.6% in *E. coli* and *K. pneumoniae*, respectively.

Hospitalized patients were treated empirically with pipericillin (alone or in combination with tazobactam), ceftriaxone, amikacin which have been shown to be among the less resistant drugs with improvement in nosocomial *K. pneumoniae* infection. While, *E. coli* mediated infections were treated with piperacillin-tazobactam, imipenem, cefepime, ceftriazone +sulbactam with reduction in infection. Tigecycline was administered to patients infected with NDM producing pathogens. All patients upon antibiotic treatment showed signs of improvement and were discharged to be followed up with routine check-up. However, there were three patient deaths amongst those reported here - a 57-year old male suffering from respiratory tract infection, a 22 year old female with acute respiratory distress syndrome and an 8 years old female with acute membranous tonsillitis who died of cardiac arrest.

DISCUSSION

This study was conducted to understand the patient recovery outcome based on the antibiotic treatment carried out for bacterial infections among hospitalized patients admitted to a tertiary care hospital in Assam, India. This also allowed for the investigation of antibiotic resistance in two most commonly circulating bacterial pathogen in Assam. It was observed that patients belonging to the age group of 45-90 years are affected by bacterial infections more often than other age groups while 19-30 years age group were more commonly affected by *Klebsiella pneumoniae*. We suspect it could be attributed to two factors (i) with age people are prone to infections due to compromised immune system and diseases that call for long term hospitalization or infection leading to nosocomial infections, and (ii) the younger generation being more robust, requiring to travel long distances are exposed to the environmental agents leading to more community based infections. The isolation rate of *E. coli* as a common causative bacterium in urinary infections is high as compared to *K. pneumoniae* which is consistent with the findings of previous studies in which *E. coli* was the predominant pathogen isolated from patients with urinary tract infections [11]. *E. coli* infections, however, are very common in all age groups which could be owing to poor personal hygiene. For the compilation of results, intermediate resistance and resistance were clubbed under resistance group as few types of β-lactamases exhibit low hydrolysis of the antibiotic with a lower MIC value or intermediate range of clearance [12]. NDM-producing isolates are resistant to different number of antibiotics tested whether co-prevalence of ESBL genes is present or not. Most of the tested clinical isolates were susceptible to imipenem suggesting possible options, but alternatively are resistant to ertapenem, meropenem and doripenem; ertapenem shows a reduced inhibition against all isolates whether they are carbapenemase-producing or not. High resistance of ertapenem could be a result of combinations of ESBLs in addition to decreased permeability or increased influx [13]. Presence of KPC gene was not observed in the given bacterial isolates.

This study allowed for the realization that administration of antibiotics that the clinical isolate shows intermediate resistance to, when used in combination with another effective antibiotic did provide relief to the patient. Piperacillin with and without tazobactam and ceftriazone with and without sulbactam were the choice of antibiotics for treatment, including imipenem and cefepime in certain cases, and the patients responded to the medication positively; some were discharged from hospitalization while the study was in progress. The MIC values for the tested antibiotics as a means of discriminating between antibiotic resistant and susceptible clinical isolate was not carried out as part of the experiments which could have helped us correlate the extent of hydrolysis of the antibiotic with the gene status of each isolate.

The challenge in dealing with resistant gram-negative pathogens is the lack of effective antibiotic agents. Cautious use of antimicrobials is therefore increasingly important as we are faced with a reduced repertoire of effective drugs. It is a not rare for a physician to have a patient die of an overwhelming infection for which there are no therapeutic options. Development of proper antibiotic policies, abstaining from the over-use of antibiotics, channeling proper and correct information among the local and rural communities,

development of infection control strategies remain crucial in curbing the dissemination of antibiotic resistant bacteria.

ACKNOWLEDGMENTS

Sample and patient details were from Department of Microbiology, Gauhati Medical College and Hospital, Guwahati, Assam, India.

Financial Disclosure: Department of Biotechnology, Government of India (Grant no. BT/213/NE/TBP/2011).

Declaration of Conflicting Interests: The authors declare that they have no conflict of interest.

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