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Detection of *blaTEM* Resistance Gene among Selected Gram-Negative Clinical Isolates in Khartoum (Sudan)

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Abstract

Background: The increased prevalence rate of carbapenem-resistant Gram-negative isolates is worrisome in clinical settings worldwide. The mortality rate associated with infections caused by these organisms is increasing over the globe.

Objective: To determine the prevalence rate of *blaTEM* gene among some Gram-negative clinical isolates in Khartoum (Sudan).

Materials and methods: This cross-sectional study was conducted in some Khartoum State hospitals during the period from April to June 2019. 110 Gram-negative clinical isolates were collected and identified following standard microbiological methods. Conventional polymerase chain reaction technique was used for the detection of *blaTEM*-gene among these isolates.

Results: *blaTEM*-gene was detected in 60 clinical isolates (54.5%) of the 110 bacterial species investigated. The gene was most commonly detected in 26 (43.3%) *Escherichia coli* strains, followed by 18 (30%) in *Pseudomonas aeruginosa* strains.

Conclusion: A high prevalence rate of *blaTEM* gene was most commonly detected among *Escherichia coli* isolates.

Keywords: Prevalence rate, *blaTEM* gene resistance, Gram-negative isolates, PCR.

Introduction

Antibiotic resistance is currently a major topic of interest for researchers and physicians. In particular, the rise of multidrug resistance in Gram-negative bacteria is now a serious challenge encountered by healthcare professionals. Resistance in Gram-negative bacteria is mainly mediated via the production of extended-spectrum β-lactamases (ESBL), ampC β-lactamases and carbapenemases. Infections with these multidrug-resistant (MDR) organisms (MDR) will thus pose therapeutic challenges; the antibiotic pipeline is drying up, and no new antimicrobial agents are anticipated in the near future to treat infections caused by these bacteria.
ESBLs were first identified in the early 1980s in Germany and had since been identified worldwide. The ESBL genes have been found in several different bacteria, including *Klebsiella pneumoniae, Escherichia coli*, *Proteus mirabilis* and *Salmonella* species. β-Lactamase producing bacteria have increasingly been reported as the cause of severe infections in intensive care and

surgical units. Infections associated with ESBL-producing bacteria include central venous line-related bacteraemia, cholangitis, intra-abdominal abscesses, peritonitis, urinary tract infections, nosocomial pneumonia and meningitis in hospitalized patients[1].

Mortality rates varying from 42% to 100% had been reported in patients infected with ESBL-producing bacteria. ESBL-producing bacteria often show cross-resistance with other groups of antibiotics, such as fluoroquinolones. Most ESBL-producing bacteria can be divided into three groups: TEM, SHV and CTX-M types. Gram-negative β-lactamases are often mediated by blaSHV, blaTEM and blaCTX-M genes. These resistant genes are located on transferable plasmids and can be freely transferred between bacteria from one region to another and from one country to the other and then spread all over the world. Initially, these bacteria contained a single ESBL-gene, but now multiple ESBL genes are commonly present in a single strain, further complicating the process of detection[2].

The TEM-type ESBL are derivatives of TEM-1 and TEM-2, and currently, TEM-3 has been discovered. The original TEM was first discovered in *E. coli* in Greece, but it spread rapidly to other genera of the gram negative-bacilli such as *Citrobacter freundii* and *Acinetobacter* spp.[(9)].

The choice of drugs for the treatment of ESBL-producing bacteria is limited to carbapenems, for example, imipenem, or alternatively, fluoroquinolones and aminoglycosides, which may be used if these antibiotics show *in vitro* activity[3].

Third-generation cephalosporins should not be used to treat serious infections with ESBL-producing bacteria, making the carbapenem the widely recognized first-choice drug. Carbapenem resistance in gram-negative bacteria has become a worldwide problem. The 2017 World Health Organization (WHO) global priority list of pathogens ranks carbapenem-resistant *Enterobacteriaceae* (CRE), carbapenem-resistant *Pseudomonas aeruginosa*, and carbapenem-resistant *Acinetobacter baumannii* in the highest priority category. Detection of carbapenems resistance gens bacteria is difficult based on routine antibiotic susceptibility testing; tests based on molecular techniques are considered the standard technique for the detection of carbapenemase genes[3]

Therefore, this study aimed to detect the *blaTEM* resistance gene among selected gram-negative clinical isolates.

Materials and methods

This was a descriptive cross-sectional study, conducted at different hospitals in Khartoum (Sudan) during the period from April to June 2019. The study was approved by the Ethical Board of Al-Neelain University, Khartoum (Sudan). Permission to collect the specimens was obtained from authorities of the different hospitals allocated in Khartoum (Sudan). The information collected had not been used for any purpose other than this study. Confidentiality of information obtained was maintained.

A total of 110 bacterial strains of different gram-negative isolates were collected from different clinical laboratories. These strains were isolated from urine, wound swabs, sputum, blood, ear swabs, and CSF samples. Furthermore, the isolates were sub-cultured on nutrient agar and re-identified using the standard, convential, microbiological techniques.

Antimicrobial susceptibility testing was performed on Muller-Hinton agar using the Kirby-Bauer

disc diffusion method according to the CLSI guidelines. Standard strains of *Pseudomonas aeruginosa* (ATCC 27853), and *Escherichia coli* (ATCC 25922) were used as controls.
Molecular detection of *BlaTEM*-gene: The DNA extraction was performed using the boiling method. 3-5 colonies were picked from a fresh nutrient agar plate and a suspension was prepared using 200 ml distilled water boiled at 100˚C for 30 minutes. The suspension was then centrifuged at 12000 rpm for 30 minutes, and the supernatant containing DNA was transferred to new Eppendrof tubes. The extracted DNA was stored at -20°C. The *blaTEM*-gene coding for carbapenem resistance was detected by the polymerase chain reaction (PCR) technique. DNA amplification of the *blaTEM*-gene was carried. The primer sequences used were:
* Forward: TCGCCGCATACACTATTCTCAGAATGA
* Reverse: ACGCTCACCGGCTCCAGATTAT.
The PCR test was performed in a 50µL volume. Thermo-cycling conditions in a thermo-cycler were as follows: 94°C for 2 min, followed by 35 cycles of 94°C for 30 sec, 60°C for 30 sec and 72°C for 30 sec, and a final extension at 72°C for 5 min. The amplified products (5µl) were separated by electrophoresis on 1.5% agarose gel and visualized by staining with ethidium bromide using a UV gel documentation system. A 445-bp PCR product was amplified with the above *blaTEM*-gene specific-primers[4].
Statistical analysis: Frequencies, mean values, standard deviations (SD) and ranges were used as descriptive statistics. The positivity of the *blaTEM*-gene was tested by means of the Mantel-Haenszel chi-square test for linear association. The statistical analysis was performed by running the SPSS/PC+ statistical package version 21, on a personal computer. A two-tailed (p of 0.05) was chosen as the cut-off for detecting statistically significant values.

Results

From the 110 clinical bacterial isolates investigated, 66 strains (60%) were isolated from females and 44 species (40%) were isolated from males. Also, 22 bacterial strains (20%) were isolated from patients aged 1-20 years, 31 bacterial strains (28%) were isolated from patients aged 21-40 years, 14 bacterial strains (13%) were isolated from patients aged 41-60 years, and 44 bacterial strains (40%) were isolated from patients aged 61-80 years. 92 bacterial species were isolated from urine specimens, 13 bacterial species were isolated from wound swabs, and only one bacterial species was isolated from ear swabs.
The commonest bacterial species isolated (47/42.7%), were *Escherichia coli* followed by (27/24.5%) *Pseudomonas aeruginosa, Klebsiella pneumoniae* (22/20%), and *Proteus mirabilis* (6/12%). The least bacterial species isolated was *Enterobacter* (1/0.9%).
The *blaTEM*-gene was detected in 60 strains (54.5%) of the 110 bacterial species. The gene was most commonly detected in *Escherichia coli* (26/43.3%), followed by *Pseudomonas aeruginosa* (18/30%) and no *blaTEM*-gene detected in *C. freundii, P. vulgaris* and *E. cloacae* (Table 1).

Discussion

As a global challenge, antimicrobial resistance in pathogenic bacteria is accompanied by high rates of mortality and morbidity. In addition, because of multidrug-resistant patterns, infections have been reported to be difficult or even impossible to treat with conventional antimicrobials.

Table (1): Distribution of *blaTEM*-gene among bacterial species investigated

Bacterial species	Positive	Negative	Total
Escherichia coli	26 (23.6%)	21 (19.1%)	47 (42.7%)
Pseudomonas aeruginosa	18 (16.3%)	9 (8.2%)	27 (24.5%)
Klebsiella pneumoniae	12 (10.9%)	10 (09.1)	22 (20.0%)
Proteus mirabilis	04 (3.7%)	2 (1.8%)	6 (5.5%)
Proteus vulgaris	0 (0%)	2 (1.8%)	2 (1.8%)
Serratia marcesence	0 (0%)	2 (1.8%)	2 (1.8%)
Citrobacter freundii	0 (0%)	3 (2.7%)	3 (2.7%)
Enterobacter cloacae	0 (0%)	1 (0.9%)	1 (0.9%)
Total	60 (54.5%)	50 (45.5%)	110 (100%)

antimicrobial susceptibility in patients with serious infections, antibiotics are widely prescribed unnecessarily[5].

Carbapenems and β-lactam antibiotics are used considerably to treat serious infections due to multidrug-resistant gram-negative bacteria. The resistance of carbapenem agents is due to the carbapenemase enzyme and the presence of other resistance mechanisms, such as ESBLs, and porin mutations. However, it is becoming a great challenge to treat infections caused by these bacteria due to its resistance against drugs and the rapid changes in the pattern of resistance.

In this study, 110 selected clinical isolates were collected from different laboratories in Khartoum and were investigated by PCR technique to detect the *blaTEM*-gene. The overall prevalence rate of *blaTEM*-gene was (60/110-54.5%).

Higher prevalence rates of *blaTEM*-gene were reported in several regions, e.g. Satir[6] (Khartoum, Sudan) reported 81.9% in 2016, and Pishtiwan[7] (Erbil, Iraq) reported 76.5% in 2019.

In contrast, lower prevalence rates of *blaTEM*-gene were reported in other regions, e.g. Ehlers[8] (Pretoria, South Africa) reported 24% in 2009, Adam[9] Khartoum, Sudan)reported 30.7% in 2018, Ojdana[10] (Poland) reported 49.1% in 2014, and Osman[11] (Port-Sudan, Sudan) reported 25% in 2017.

Variation in prevalence rates could be due to sample size, high nosocomial resistance of pathogens, and methods of hospital hygiene applied for antibiotics abuse, poor infection control, and spread of hospital-acquired resistance genes into the community and vice versa.

In the present study, *blaTEM*-genes were highly detected (26/43.3%) among *E. coli* species, followed by *Pseudomonas aeruginosa* (18/30%), *Klebsiella pneumoniae* (12/10.9%), *Proteus*

mirabilis (4/3.7%). No *blaTEM*-gene was detected among *Citrobacter freundii*, *Proteus vulgaris Enterobacter cloacae,* and *Serratia marcesence.* Pishtiwan (2019)[8] reported high *blaTEM*-gene prevalence rates (81% and 64.7%) among *E. coli* and *Klebsiella pneumoniae* respectively.
Conclusion: A high prevalence rate of *blaTEM* gene was most commonly detected among *Escherichia coli* isolates.

References

1. Winkokur, L; Canton, R; Casellas, M and Legakis, N. Variations in the prevalence of strains expressing an extended-spectrum β-lactamase phenotype and characterization of isolates from Europe, the Americas and the Western Pacific region. *Clin Infect Dis.* 2001; 32: 94-103.
2. Samaha-Kfoury, N and Araj, F. Recent developments in β-lactamases and extended-spectrum β-lactamases. *BMJ*. 2003; 327: 1209-1213.
3. Pitout, D; and Laupland, B. Extended-spectrum β-lactamase producing Enterobacteriaceae: an emerging public health. *Microbiol Infect Dis*. 2008; 8: 159–16
4. Akova, M. Epidemiology of antimicrobial resistance in bloodstream infections. *Virulence,* 2016; 7:252-266.
5. Monstein, J; Östholm-Balkhed, A; Nilsson, V. *et al.* Multiplex PCR amplification assay for the detection of blaSHV, blaTEM and blaCTX-M genes in *Enterobacteriaceae.* 2007;APMIS; 115: 1400-1408.
6. Satir, S; Elkhalifa, A; Ali, M. *et al.*. Detection of carbapenem resistance genes among selected Gram negative bacteria isolated from patients in-Khartoum State, Sudan. *Clin Microbiol,* 2016; 5:(6):2-4.
7. Pishtiwan, A; and Khadija, K. Prevalence of blaTEM, blaSHV, and blaCTX-M genes among ESBL-producing *Klebsiella pneumoniae* and *Escherichia coli* isolated from thalassemia patients in Erbil, Iraq. *J Hematol Infect Dis* 2019, 11(1): e2019041,
8. Ehlers, M; Veldsman, C; Makgotlho, E. *et al.* Detection of blaSHV, blaTEM and bla CTX-M antibiotic resistance genes in randomly selected bacterial pathogens from the Steve Biko Academic Hospital FEMS. *Immunol Med Microbiol.* 2009; 9: 191-196
9. Adam, M; and Elhag, W. Prevalence of metallo-β-Lactamase acquired genes among carbapenems susceptible and resistant Gram-negative clinical isolates using multiplex PCR, Khartoum hospitals, Khartoum Sudan. *BMC Infectious Diseases*. 2018;18:668-672.
10. Ojdana, D; Sacha, P; Wieczorek, P. *et al.* The Occurrence of blaCTX-M, blaSHV, and blaTEM genes in extended-spectrum β-Lactamase-positive strains of *Klebsiella pneumoniae, Escherichia coli*, and *Proteus mirabilis* in Poland. *Intern J Antibi.* 2014; 2014:1- 7.
11. Osman, A; Hashim, S; Musa, M; and Tahir, O. Detection of CTX-M, TEM and SHV genes in Gram-negative bacteria isolated from nosocomial patients at Port-Sudan Teaching Hospital. *European Journal of Clinical and Biomedical Sciences.* 2017;3(6):101-8.