**Extended Spectrum Beta-Lactamase Producing Enterobacteriaceae: Hospital-Acquired Urinary Tract Infections, Khartoum-Sudan**

Omnia M Hamid1*, Samia A Gumaa2, Al Amin Ibrahim3 and Magdi Bayoumi4

1Faculty of Medical Laboratory Sciences, Department of Medical Microbiology, University of Medical Sciences & Technology, Sudan
2Professor of Microbiology, Head of Microbiology Department, Royal Care International Hospital (RCIH), Sudan
3Associate Professor of Medical Microbiology, Dean of the Faculty of Medical Laboratory Sciences, University of Khartoum, Sudan
4Associate Professor of Pathology, Director, Ibrahim El Zain Training Centre (IBRAZ), Ibn Sina University, Khartoum Sudan

**Abstract**

**Background:** The family Enterobacteriaceae are the most common causative agent of urinary tract infection (UTI) pathogen among both hospital and community patients. Antibiotic resistance among uropathogens has become a major public health problem that due to easy resistant spread and transfer resistant gene within gram negative bacteria, also might led to limitations of treatment options. This study was aimed to determine the prevalence of Extended Spectrum ß-Lactamase (ESBL)-producing Enterobacteriaceae isolates from urine samples in patients admitted in Academy Charity Teaching Hospital (ACTH) by phenotypic detection, Khartoum State, Sudan.

**Methods:** A descriptive; cross-sectional study was conducted on 350 Clean-catch, mid-stream urine samples (MSU) from both gender (female and male) patients hospitalized in Academic Charity Teaching Hospital with sign and symptom of UTI. The gram-negative isolates were identified and screened by Kirby-Bauer disc diffusion method. Double-Disc Synergy Test (DDST) was performed for detection and confirmation of ESBLs. Socio-demographic and clinical data were obtained from each participant using close-end questionnaires with palace to sign or stamp (a way to protect patients confidentiality) and check list for practical follow-up work and record.

**Results:** A total of 104 MSU samples were diagnosed to have growth of Enterobacteriaceae species for significant bacteriuria (≥ 10⁵/CFU); Escherichia coli was the most frequently isolated organism (43.3%); followed by Klebsiella pneumoniae (22.1%), Proteus mirabilis (11.5%), Enterobacter aerogenes (7.7%), Klebsiella oxytoca and Serratia marcescens (5.8%) for each and (1.9%) of Enterobacter cloacae and Citrobacter freundii for each. ESBL was detected in 67 (64.4%) isolates including 82.2% (37/45) of E. coli resistant to cefotaxime (30 µg) in screening ESBL detection. Double-disk synergy test (DDST); ESBL confirmatory method was performed on Enterobacteriaceae isolated species 23.1% (24/104) was ESBL producing uropathogens, which included 13 isolates of E. coli (28.9%) and 8 of K. pneumoniae (34.8%).

**Conclusions:** The high prevalence of ESBL producing Enterobacteriaceae among hospitalized patients with UTI, indicates to develop a monitoring system of the drug resistance for scientific-based selection of antibiotic therapy and requires hospital policies on empirical use of antibiotic. Routine ESBL phenotypic detection testing and subsequent antibiogram with disk diffusion method could be perform as easy and costless to third countries.

**Keywords**

Extended-spectrum ß-lactamases-Urinary tract infection, Enterobacteriaceae

**Abbreviations**


---

Copyright: © 2019 Hamid OM, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
Background

Gram-negative bacteria (GNB) mainly Enterobacteriaceae family are predominately in hospital-acquired urinary tract infections; urinary tract infection (UTI) is one of the most common GNB infections acquired either from community or hospital settings and affecting all age groups, sexes and might asymptomatic or symptomatic depending on the pathogenesis of infection [1]. Also, most of bacteriuria cases are generally asymptomatic, and an effective management is removing the catheter rather than antibiotic treatment. Worldwide; it is the most common nonsurgical hospital acquired infection (HAI) and the second most common healthcare associated infection which constitutes 40-50% of all hospital infections also as the second most common reason for empirical antibiotic treatment, UTI is a major driver of antibiotic usage globally [2].

Multi-drug resistance GNB are common challenge to patient-hospital safety and protection control due to limit treatment options [3]. These Enterobacteriaceae family strains have an efficient mechanism by up-regulating or acquiring genes that code for drug resistance [2], production of β-lactamases is the primary mechanism of β-lactam resistance in these pathogens [4]. Extended spectrum β-lactamase (ESBL) can confer resistance to penicillins, first-, second-, and third-generation cephalosporins, and aztreonam; they are usually hindered by β-lactamase inhibitors such as clavulanic acid. Most ESBLs can also hydrolyze fourth-generation cephalosporins (e.g., ceftazidime or ceftepime). Furthermore, Enterobacteriaceae harboring ESBLs are often co-resistant to fluoroquinolones, aminoglycosides, and trimethoprim/sulfamethoxazole [5]. In Gram negative bacteria these enzymes remain in the periplasmic space, where they attack the antibiotic before it can reach its receptor site. ESBL is the first plasmid mediated β-lactamase was described in early 1960 mainly among Enterobacteriaceae family and other GNB [6,7]. The emergence of resistance to extended-spectrum cephalosporins remains challenging; these agents are often first-line therapy [8,9]. Unfortunately, the plasmids carrying ESBL genes often carry resistance determinants targeting fluoroquinolones as well. Where the sensitivity and specificity in traditional susceptibility tests are unable to detect ESBLs; has led to the search for an accurate and cost-effective test to detect the presence of ESBL in hospitalized patients. The aim of this study was to determine the prevalence of ESBL producing hospital Enterobacteriaceae in urinary tract infection.

Methods

Study design

A facility-based descriptive cross-sectional study was conducted. The research was conducted during the period of December 2007 to July 2008 in the urology department of Academic Charity Teaching Hospital (ACTH) located in Khartoum State, Sudan. All females and males participants, aged 20 to 65 years, admitted in the urology department of ACTH for UTI were included in the study. Were excluded patients with primary negative urine culture and whose urine culture grew negative for Enterobacteriaceae, day-care patients, pregnant women, diabetic and patients hospitalized for other underline infection not related UTI.

Collection of samples

A total of clean-catch midstream urine (MSU) samples (n = 350) were collected, from males (n = 157) and females (n = 193) in-patients with a mean age of 40 years diagnosed with recurrent urinary tract infection, in sterile and screw capped disposable containers. Patients aged < 18 years considered as pediatric population and having specific treatment guidelines, were excluded. Specimens such as a nephrostomy, ileal conduit, extra prostatic secretion, suprapubic aspirate, or bag specimens were also excluded as they represent distinct clinical scenarios. Well defined guidance was provided to patients to avoid contamination of urine samples. All the collected specimens were properly labeled, and data were recorded through a standardized questionnaire.

Isolation and identification of bacterial species

Uncentrifuged urine samples were processed by calibrated loop onto blood agar and MacConkey agar plates, immediately or within 2 hours from collection. Plates were incubated aerobically at 37 °C (24 hours) overnight; the cut-off value of ≥ 10^5 CFU/mL was considered as significant growth [10]. Media culture and biochemical’s test media, and API 20E (bio-Merieux, Marcy-l’Etoile, France) for biochemical confirmations were used according to the manufacturer’s instructions.

Antimicrobial susceptibility test

For screening ESBL production the antimicrobial susceptibility test was performed using Kirby-Bauer disc diffusion technique as recommended by the Clinical and Laboratory Standards Institute [11] (CLSI, 2011). Mueller-Hinton agar culture medium (Himedia, India) was inoculated by a direct saline suspension of isolated colonies with turbidity of 0.5 McFarland. Then antibiotic disks (Oxoid, UK), cefotaxime (30 μg), ceftazidime (30 μg), cefpodoxime 10 μg and aztreonam 30 μg were placed on the agar surface at a distance of 30 mm (center to center) from each other. After 18 hours of incubation at 37 °C, results were interpreted by measuring the inhibition zone diameter around each disk. According to CLSI criteria, an inhibition zone of ≥ 27 mm for aztreonam and ≥ 22 mm for ceftazidime, cefotaxime and cefpodoxime indicated that the strain possibly produced ESBL. E. coli ATCC® 25922 was used as control strains for both.

Confirmatory test for ESBL production

The Double-Disc Synergy Test (DDST) by Jarlier [12] was performed for all isolates resistant to at least two of applied antibiotics.
Table 1: Distribution of significant and no significant growth of *Enterobacteriaceae* from inpatients urine samples.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Number (%) of urine samples</th>
<th>Enterobacteriaceae isolates</th>
<th>Odd ratio</th>
<th>Pearson Chi-square value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No significant growth (&lt; 10⁵ cfu/mL)</td>
<td>Significant growth (&gt; 10⁵ cfu/mL)</td>
<td>Value</td>
<td>95% CI</td>
</tr>
<tr>
<td>Female</td>
<td>193 (55.14%)</td>
<td>141 - 73.06%</td>
<td>52 - 26.94%</td>
<td>0.74</td>
<td>0.47</td>
</tr>
<tr>
<td>Male</td>
<td>157 (44.86%)</td>
<td>105 - 66.87%</td>
<td>52 - 33.12%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>350 (100.00%)</td>
<td>246 - 70.29%</td>
<td>104 - 29.71%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

%: Percentage; CI: Confidence interval; df: Degree of freedom; *: Insignificant.

Table 2: Frequency and distribution of *Enterobacteriaceae* isolates from urine samples (n = 104).

<table>
<thead>
<tr>
<th>Bacterial isolates</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>45 (43.3%)</td>
</tr>
<tr>
<td><em>K. pneumonia</em></td>
<td>23 (22.1%)</td>
</tr>
<tr>
<td><em>P. mirabilis</em></td>
<td>12 (11.5%)</td>
</tr>
<tr>
<td><em>E. aerogenes</em></td>
<td>8 (7.7%)</td>
</tr>
<tr>
<td><em>K. oxytoca</em></td>
<td>6 (5.8%)</td>
</tr>
<tr>
<td><em>S. marcescens</em></td>
<td>6 (5.8%)</td>
</tr>
<tr>
<td><em>E. cloacae</em></td>
<td>2 (1.9%)</td>
</tr>
<tr>
<td><em>C. freundii</em></td>
<td>2 (1.9%)</td>
</tr>
<tr>
<td>n (%)</td>
<td>104 (29.7%)</td>
</tr>
</tbody>
</table>

Keynote: n = total number of isolates.

Antimicrobial susceptibility testing of the isolates

The susceptibility pattern of isolated ESBL-producing *Enterobacteriaceae* species, determined by disk diffusion test revealed that isolates with higher resistant 64.4% (67/104) were towards cefotaxime and cepodoxime had lowest resistance 32.7% (34/104) of isolates. Cefpodoxime was the most effective antibiotics on *E. coli* and *K. pneumoniae* and other species tested. On the other hand, *Enterobacteriaceae* strains showed high resistance to cefotaxime antibiotics, which could be used as clue antibiotic for ESBL screening test (Table 3).

Confinatory tests for ESBL detection

The Double-Disc Synergy Test (DDST) by Jarlier [12] was observed for cefotaxime with Amoxicillin/clavulanic acid against the isolates, 24 (23.1%) of *Enterobacteriaceae* isolates were ESBL-positive to CTX/AMX, CAZ/AMX 18 (17.3%), AZT/AMX (14.4%) and Low ESBL production was detected using SPD/AMX 6 (5.8%). The DDDT method is significant with p value 0.000 at p < 0.05 and chi-square statistic is 32.8251, which indicated that the cefotaxime disc it is the better disc use for confirmation (Table 4).

Discussion

As urinary tract infection is commonest disease among hospitalized patients among both gender, its diagnosis and treatment have important consequences for patients' health, development of antibiotics resistance, spreading of multidrug resistant strains and health care costs [13]. Among hospitalized patients with UTIs at this public (ACTH), teaching hospital in Khartoum-Sudan, the prevalence of UTI due to *Enterobacteriaceae* was 104 (29.7%) out of 350 patients.

Their age ranged from 20 to 65 years, with average of 40 years ± 11.64. Where 104 urine samples had growth of *Enterobacteriaceae* equally distributed in gender; there was no significant difference in the gender and *Enterobacteriaceae* growth between significant and non-significant UTI among inpatients selections with p-value 0.4533 (Table 1). The predominating pathogen was *E. coli* 43.3% (n = 45), followed by *K. pneumoniae* 22.1% (n = 23). Table 2; summarize the distribution of the various *Enterobacteriaceae* species isolated.

Of 104 isolated *Enterobacteriaceae* Species, 24 (23.1%) were ESBL-positive which included 13 isolates of *E. coli* (54.2%), 8 isolates of *K. pneumoniae* (33.3%) and 3 isolates of other species (4.2%).
Extended Spectrum Beta-Lactamase Producing Enterobacteriaceae: Hospital-Acquired Urinary Tract Infections, Khartoum-Sudan

Table 3: 3rd generation cephalosporins antibiotic resistance pattern of isolated Enterobacteriaceae determined by disk diffusion (ESBL screening) test.

<table>
<thead>
<tr>
<th>Bacterial isolates</th>
<th>Cefotaxime</th>
<th>Aztreonam</th>
<th>Ceftazidime</th>
<th>Cefpodoxime</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resistant, n (%)</td>
<td>Resistant, n (%)</td>
<td>Resistant, n (%)</td>
<td>Resistant, n (%)</td>
</tr>
<tr>
<td><em>E. coli</em> (n = 45)</td>
<td>37 (82.2%)</td>
<td>38 (84.4%)</td>
<td>30 (66.7%)</td>
<td>16 (35.6%)</td>
</tr>
<tr>
<td><em>K. pneumoniae</em> (n = 23)</td>
<td>21 (91.3%)</td>
<td>17 (73.9%)</td>
<td>19 (82.6%)</td>
<td>13 (56.5%)</td>
</tr>
<tr>
<td><em>P. mirabilis</em> (n = 12)</td>
<td>4 (33.3%)</td>
<td>3 (25%)</td>
<td>1 (8.3%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><em>E. aerogenes</em> (n = 8)</td>
<td>1 (12.5%)</td>
<td>3 (37.5%)</td>
<td>2 (25%)</td>
<td>2 (25%)</td>
</tr>
<tr>
<td><em>K. oxytoca</em> (n = 6)</td>
<td>0 (0%)</td>
<td>2 (33.3%)</td>
<td>0 (0%)</td>
<td>1 (16.7%)</td>
</tr>
<tr>
<td><em>S. marcescens</em> (n = 6)</td>
<td>2 (33.3%)</td>
<td>3 (33.3%)</td>
<td>3 (50%)</td>
<td>1 (16.7%)</td>
</tr>
<tr>
<td><em>E. cloacae</em> (n = 2)</td>
<td>1 (50%)</td>
<td>0 (0%)</td>
<td>1 (50%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><em>C. freundii</em> (n = 2)</td>
<td>1 (50%)</td>
<td>1 (50%)</td>
<td>1 (50%)</td>
<td>1 (50%)</td>
</tr>
<tr>
<td>Total (n = 104)</td>
<td>67 (64.4%)</td>
<td>66 (63.5%)</td>
<td>57 (54.8%)</td>
<td>34 (32.7%)</td>
</tr>
</tbody>
</table>

*3rd GC: Third generation cephalosporins.

Table 4: Results of DDST method for isolated ESBL-producing Enterobacteriaceae resistant to 3rd GC antibiotics in ESBL screening test.

<table>
<thead>
<tr>
<th>Number (%)</th>
<th>CTX/AMX</th>
<th>CAZ/AMX</th>
<th>AZT/AMX</th>
<th>CPD/AMX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial isolates</td>
<td>ESBL, n (%)</td>
<td>ESBL, n (%)</td>
<td>ESBL, n (%)</td>
<td>ESBL, n (%)</td>
</tr>
<tr>
<td><em>E. coli</em> (n = 45)</td>
<td>13 (28.9%)</td>
<td>9 (20%)</td>
<td>6 (13.3%)</td>
<td>2 (4.4%)</td>
</tr>
<tr>
<td><em>K. pneumoniae</em> (n = 23)</td>
<td>8 (34.8%)</td>
<td>4 (17.4%)</td>
<td>6 (26.1%)</td>
<td>4 (17.4%)</td>
</tr>
<tr>
<td><em>P. mirabilis</em> (n = 12)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><em>E. aerogenes</em> (n = 8)</td>
<td>1 (12.5%)</td>
<td>2 (25%)</td>
<td>1 (12.5%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><em>K. oxytoca</em> (n = 6)</td>
<td>1 (16.7%)</td>
<td>1 (16.7%)</td>
<td>1 (16.7%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><em>S. marcescens</em> (n = 6)</td>
<td>1 (16.7%)</td>
<td>2 (33.3%)</td>
<td>1 (16.7%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><em>E. cloacae</em> (n = 2)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><em>C. freundii</em> (n = 2)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Total</td>
<td>24 (23.1%)</td>
<td>18 (17.3%)</td>
<td>15 (14.4%)</td>
<td>6 (5.8%)</td>
</tr>
</tbody>
</table>

*CAZ: Ceftazidime; CPD: Cefpodoxime; AZT: Aztreonam; CTX: Cefotaxime; AMX: Amoxicillin.

this prevalence is similar to the published data in Chicago (73.5%) [15] and Nigeria (81.7%) [16]. The differences observed between the studies can be due to the methods and resistance patterns could be related to environmental factors and antimicrobial susceptibility test used. In this study, the number of male patients to female patient’s ratio was 1:1.2. And among positive cases, the ratio was 1:1.27. Females were higher in both cases which was natural enrolment bias. Females are more frequently affected due to colonization of urethra with colonic Gram-negative bacteria because of its proximity to anus and short length of urethra as patient’s gender is risk factor of UTI [17].

Antimicrobial resistance has become a serious worldwide public health issue. Infections caused by drug resistant bacteria are responsible for increased morbidity, mortality, prolonged hospital stay and increased government hospital costs. The prevalence of Enterobacteriaceae ESBL producing was (23.1%) in our research, as appeared in Khartoum 2007 that give us alarming at that time to search on prevalence of ESBL producer. A recent study in Sudan 2014 [18] reported 65%, higher than our 2008 findings. The increase may be related to the lack of guidelines for treatment of UTI, and the misuse of antibiotics. The prevalence of ESBL producing Enterobacteriaceae varies geographically worldwide from (< 1% to 74%) [19]. It is reported as (10%) [20] in East Europe, (3.5%) [21] in a Canadian study and (20-48.8%) [22] in Asia. Within the Arabian Gulf region, various ESBL prevalence were reported (7.5%) [23] in Kuwait, (41.0%) [24] in United Arab Emirates and (22.0%) [25] in Saudi Arabia. In the African region [26] Algeria, Egypt and Nigeria reported 31.4%, 19.0%, and 12.8% hospital acquired ESBL producing Enterobacteriaceae respectively. These findings impose the need of suitable use of antibiotics based on accurate bacteriological testing along with appropriate guidelines.

Increasing unreasonable and miss-use of antibiotics, sales of substandard antibiotics and transmission of drug resistant bacteria among hospitalized patients may be responsible for the rise in antibiotic resistance among the bacteria. By screening method 67 (64.4%) Enterobacteriaceae species isolates were found to be resistant to cefotaxime, followed by aztreonam 63.5%, ceftazidime 54.8% and low resistant was 32.7% to Cefpodoxime. This finding is lower than those obtained by Kamlesh K [27] who reported cefotaxime (74.74%) and ceftazidime (83.16%). Empirical antibiotic selection should be
based on knowledge of the local prevalence of bacterial organisms and antibiotic sensitivities, because resistance patterns may vary according to the region of concern.

On the other hand 82.6% of *E. coli* were resistant to CTX and 91.3% of *K. pneumoniae* ESBL positive isolates in the screening test from urine in-patients was the most common ESBL producer, This percentages is considered high when compared to published studies in India; as described in 2005 [28] were 82.6% of *K. pneumoniae*, and 66.7% of *E. coli* and 19.8% *E. coli* and 31.8% *K. pneumoniae* resistant to CAZ and in 2008 [29]. Lower prevalence’s were reported elsewhere in Korea (4.8%), Taiwan (8.5%) and Hong Kong (12.0%) [30-32]. However, *E. cloacae* (20 ESBL positive isolates) had a high prevalence than other classic producers (33.3% of *P. mirabilis* were resistant to CTX &50% of *S. marcescens* was resistant to CAZ). The same observation was made in 2008 [29].

The ubiquity of ESBL-producing *E. coli* was observed highlighting the importance of regular surveillance of ESBL producing clinical isolates in clinical samples to minimize increasing of multi-drug resistance strains among hospital and community, to prevent the ineffectiveness of antimicrobial agent for good health practices [33]. Our study revealed that the ESBLs producing uropathogenic *E. coli* isolates were 28.9%. This finding is almost the same as the study reported (26.87%) *E. coli* by Kamlesh [27]; our results are slightly higher than data published by various authors such as [34-36] who reported ESBLs producer 24.5%, 19.02% and 21.4% respectively. Other authors reported higher prevalence of *E. coli* ESBL producer of 61.2%, 35% from Suadia Arabia [25] and 42.31% Khartoum [18] respectively. The variability of the reported prevalence of UTI with ESBL producing *Enterobacteriaceae* in inpatients may be related to both the antibiotic and the microorganism tested across countries.

ESBL-producing *K. pneumoniae* as an important urinary pathogen in this group of patients. However, *K. pneumoniae* also seems to represent an important urinary pathogen in young females as supported by 34.8% is the high isolation rates in this group of patients observed in our study and by This result is in line with findings reported in a previous study in Italy [37]. Conversely, the high rates for non-ESBL mediated cefpodoxime resistant *Enterobacteriaceae* isolates 5.8% may be due to their different mechanisms for resistance. This further limits the therapeutic options available to treat these infections.

The ESBLs-producing *Enterobacteriaceae* were most frequent in older age group in our study; also associated with risk factors such as prolonged hospitalization, previous antibiotic use, and presence of invasive devices [38]. According to the majority of isolates were from patient’s age between 40 to 70 years, as our findings revealed a high level of the ESBL-producing *Enterobacteriaceae* in hospital acquired UTIs in patients of aged > 40 years, other studies also pointed that ESBL isolates are encountered more frequently in the elderly [38,39].

The high resistant to antibiotic in our community may be due to empirical use of drug from patients relatives plus easy to get medication from pharmacist, and mainly most of the older inpatients used traditional and herbal medication that might be one of the risk factors that play a major role in develop antimicrobial resistant not only to cephalosporin also extend to other antimicrobial type and in 2008 there are no studies showing a rate of ESBL producing *Enterobacteriaceae* in Khartoum state which gave us highlighting to search at that time according resistant observation among *Enterobacteriaceae* during daily hospital report.

**Conclusion**

At that time 2008 this is the first study conducted to determine the prevalence of *Enterobacteriaceae* producing ESBL among hospitalized patients with UTI, with the effect of gender and age on its prevalence, and highlight to screen ESBL with DDDT method which is easy to perform and cost less to our community as a routine antimicrobial susceptibility test in hospitals microbiology laboratories, at least for antibiotic-resistant microorganisms, for best practice in treatments of UTI. ESBL producing *Enterobacteriaceae* was observed in 23.1% of the total isolates.

**Conflict of Interest Statement**

We declare that we have no conflict of interest.

**Acknowledgments**

A deep appreciation goes to Dr. Mounkaila Noma for helping us throughout paper editing & formatting.

**Funding**

The research was fully funded by the author in the frame of a master degree submitted to the Faculty of Medical Laboratory Sciences at the University of Medical Sciences and Technology. No funding source.

**Availability of Data and Materials**

All relevant data is reported in the manuscript and attached document upon request.

**Authors Contributions**

Omnia M Hamid developed the study design, collected, processed samples, analyzed samples and prepared (M.Sc research degree), Samia A Guma thesis supervisor, and Al Amin Ibrahim was samples process and lab test technique reviewer, Magdi Bayoumi approved the final thesis and manuscript writing.

**Ethical Consideration**

Well verbal informed the participant; participation was voluntary and anytime had the right to withdraw from the study, and the questionnaire and consent form of the participants agreement was either to sign or stamp (because some patients uneducated) on the research tool on compilation of the data collection, process or published. The research project was approved by the research committee (SUMASRI-international Review Board); Faculty of Medical Laboratory Sciences, University of Medical Sciences and Technology, Khartoum-Sudan.
Competing Interests
The authors declare that they have no competing interests.

Consent for Publication
All authors read and approved to the submitted version of this manuscript.

References


