Prevalence of Carbapenemase Encoding Genes and Antimicrobial Resistance Pattern of Enterobacteriaceae Isolated from Hospitalized Patients in Khartoum State, Sudan

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Abstract Carbapenem-resistant Enterobacteriaceae strains have been responsible for an increasing number of hospital-acquired infections globally. This study aimed to determine the prevalence of carbapenemase-producing Enterobacteriaceae and their frequency of antimicrobial-resistant patterns among hospitalized patients across three Khartoum State Teaching Hospitals, Sudan. Materials and Methods. A cross-sectional study was conducted at Khartoum State Teaching Hospitals from April 2018 to October 2019. A total of 384 non-duplicative Enterobacteriaceae strains were isolated from 1062 clinical samples obtained from hospitalized patients receiving treatment across three main teaching hospitals. The samples were cultured into a MacConkey agar plate. The Enterobacteriaceae strains were differentiated by specific colony color and again by biochemical test. Antimicrobial susceptibility testing was performed by the disc diffusion method. The minimum inhibitory concentration (MIC) of imipenem and meropenem was performed by the agar dilution method. Multiplex polymerase chain reaction (PCR) was performed to investigate the presence of carbapenemase-encoding genes. Data analysis was carried out using SPSS version 21. Results. Of the 36.2% (384/1062) nonduplicate of Enterobacteriaceae strains isolated from clinical samples, 122 (31.8%) were carbapenemase-producing Enterobacteriaceae (CPE). Of these isolates, 37 (30.3%) harbored the blaIMP followed by; 29 (23.8%) blaNDM, 21 (17.2%) blaOXA-48, 6 (4.9%) blaGES, 5 (4.1%) were blaKPC, 3(2.5%) blaGIM-1, 2(1.6%) blaVIM and 1 (0.8%) blaSIM-1, while the remaining 19(15.6%) isolates carried combinations carbapenemase blagenes. The most predominant CPE strains were Escherichia coli 40 (32.8%) followed by Klebsiella pneumoniae 24 (19.7%) and Enterobacter aerogenes 14 (11.5%). Most of the CPE isolates were isolated from wound swab 40 (32.8), sputum 33 (27.0), and urine 22 (18.0) samples. Furthermost strains showed high resistance rates (>70%) to the antibiotics tested. Resistance to amikacin, tetracycline, co-trimoxazole, and nalidixic acid was 36.9%, 43.4%, 62.3%, and 63.9%, respectively and 82.8% of CPE strains were susceptible to colistin. The detection of blagenes carbapenemases in CPE strains had a significant effect on both imipenem and meropenem MICs. Conclusion. The most prevalent carbapenemase-producing blagenes among clinical Enterobacteriaceae clinical isolates from the three Khartoum state regions were blaIMP, blaNDM, and blaOXA-48. In contrast, the propensity of the multidrug-resistant profile that has been associated with producing carbapenemase blagenes is alarming. Therefore, it is very important to establish a routine screening of carbapenemase-producing blagenes in clinical isolates to prevent the dissemination of resistant strains among both inpatients and outpatients in hospital settings.

Keywords: carbapenemase, Enterobacteriaceae, Khartoum, prevalence, resistance

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1. Introduction

Enterobacteriaceae are a family of bacteria that encompass many bacteria that are commonly isolated from clinical cultures, including Escherichia coli, Klebsiella spp., and Enterobacter spp. they are frequently implicated in community-acquired infections and carbapenem-resistant Enterobacteriaceae (CRE) have the potential to be transmitted from current healthcare-exposed patients to the community [1]. Organisms express
resistance to carbapenems through several mechanisms e.g. altered penicillin-binding proteins (PBPs) and porin function, β-lactamases production, and efflux pumps [2,3]. Though, the resistance rate against carbapenem has increased dramatically due to its frequent application in recent years and is mainly mediated by the production of carbapenem- hydrolyzing enzymes [4]. There is a vast diversity of β-lactamases today, and the most worrisome are the carbapenemases as it encoded by a highly transmissible gene [2].

Enterobacteriaceae producing these mobile carbapenemases are termed "Carbapenemase-producing Enterobacteriaceae (CPE)", distinguishing them from "Carbapenem-resistant Enterobacteriaceae (CRE)", which refers to Enterobacteriaceae which are phenotypically resistant to carbapenems, by any mechanism (e.g. porin mutations, efflux pumps, or carbapenemase production) [2,3]. Carbapenemase production is especially problematic when encountered in members of the family Enterobacteriaceae. Due to their ability to readily spread and colonize patients in healthcare environments [5]. The emergence of acquired carbapenemase genes in clinical settings has become a major clinical concern because these bacteria in this family are resistant to nearly all antibiotics classes of antibiotics, leaving more toxic or less effective treatment options [6]. Increases in CPE have been reported internationally, with endemic situations in the USA, India, and Greece and predictions of forthcoming worldwide epidemics [7]. The World Health Organization (WHO) lists Enterobacteriaceae among critical antibiotic-resistant as a global serious threat to public health, with (CPE) identified as a growing challenge [8].

Carbapenemase producing-Carbapenem resistant Enterobacteriaceae can produce a large variety of carbapenemases [9]; the most common in Enterobacteriaceae strains is the production of β-lactamases, including enzymes of Ambler class A enzymes consisting; Klebsiella pneumoniae carbapenemase (KPC) and Guiana extended-spectrum (GES) Plasmid encoded carbapenemases, Amble class B enzymes consisting; Metallo-β-lactamases (MBLs) mainly New Delhi Metallo-β-lactamase (NDM), imipenemase (IMP), Verona integron encoded Metallo-β-lactamase (VIM), German imipenemase (GIM-1), and Seoul imipenemase (SIM-1) types, and third clinically relevant are Class D are oxacillin hydrolyzing enzymes (OXA) the carbapenemases are six widespread of OXA-48-like; OXA-48 being the most widespread and the remaining variants are OXA-162, OXA-163, OXA-181, OXA-204 and OXA-232 [5,9,10].

Studies in Africa and Sudan [11] (e.g., Egypt, South Africa, and Morocco) have reported increasing CPE among hospitalized patients [12,13,14]. The carbapenemase genes in Enterobacteriaceae are associated with mobile genetic elements such as plasmids or transposons, thereby facilitating infection outbreaks and leading to therapeutic problems for the treatment of hospitalized patients in our region, as well as other countries [15].

The current study aimed to investigate the prevalence of carbapenemase-producing blagenes, as well as to detect the frequency of antimicrobial-resistant patterns in Enterobacteriaceae isolates obtained from hospitalized patients across three Khartoum state teaching hospitals, Sudan 2018-2019.

2. Materials and Methods

2.1. Study Design and Settings

This was a prospective cross-sectional hospital-based study conducted at three main Teaching Hospitals in Khartoum State, Sudan. The Khartoum state is formed by three localities; which were purposively selected in Khartoum locality (Ibrahim Malik Teaching Hospital), Omdurman locality (Omdurman Teaching Hospital), and Khartoum North locality (Baheri Teaching Hospital). In each of these hospitals, five wards were included; medical, inflamed wound, respiratory, surgical, and renal. With an estimated total population of Khartoum State 6,846,460 in 2019 projected from the (2008) population 5,274,321 and the gross rate of 2.4% according to [16] in 2016 the prevalence of CPE is 63% Khartoum state which represents 3,450,616 people who attended hospital in Khartoum state base on this estimated sample size of 384 (358 with adding error 25) was calculated based on the statistical formula of Fisher for calculating sample size [17]:

\[
N = \frac{Z^2P(1-P)}{d^2}
\]

where N is the minimum sample size for a statistically significant survey, Z is normal deviant at the portion of 95% confidence interval = 1.96 and P is prevalence value of CPE (63%), and d is margin of error acceptable or measure of precision = 0.05. this population was reached three selected hospitals by screening 1062 clinical specimens through 18 months of data collection (from April 2018 to October 2019).

2.2. Data Collection

Data was collected based on feasibility and permission reason of participants to be in this study. The clinical specimens were collected from hospitalized patients and a direct interviewing standardized questionnaire was filled to determine the patient's characteristics including age, gender, specimen types, isolation site, and hospitalization ward and ward to associate with CPE blagene and non-CPE.

2.3. Bacterial Isolates

A total of 1062 non-duplicate various clinical specimens (wound swab, urine, sputum, urine catheter, blood, stool, pleural fluid, and other body fluid) were transported to the Research Laboratory of Graduate College at the University of Medical Sciences and Technology-UMST-Sudan. The clinical significance of all isolates was determined at each inpatient ward based on the patient's suited clinical and laboratory findings. From these specimens, 664 non-duplicated and clinically significant bacterial isolates were obtained. Identification of the Enterobacteriaceae species was performed using biochemical tests and unidentified
species confirmed by the API20E (bioMe´rieux, Marcy l’Etoile, France). All of the strains were stored at −80°C in brain heart infusion broth with 15% glycerol until further use.

2.4. Antimicrobial Susceptibility Testing

A total of 18 clinically relevant antibiotics were tested using the disc diffusion method (Kirby-Bauer’s) and the inhibition zone diameters were measured by (mm) according to Clinical and Laboratory Standards Institute (CLSI) guidelines M100 27th [18]. These antimicrobial discs were amoxicillin/clavulanic acid (30μg), ampicillin (10μg), cefotaxime (30μg), ceftazidime (30μg), ceftriaxone (30μg), aztreonam (30μg), imipenem (10μg), meropenem (10μg), ertapenem(10μg), gentamicin (10μg), tobramycin (10μg), amikacin (30μg), tetracycline (30μg), ciprofloxacin (5μg), norfloxacin (10μg), nalidixic acid (30μg), co-trimoxazole (25μg) and colistin (10μg) were obtained from HiMedia Laboratories (India). The results for the antimicrobial susceptibility test strain were interpreted as (S) susceptible, (I) intermediate, or (R) resistant by comparing the results to the CLSI guidelines, the carbapenems resistant Enterobacteriaceae (CRE) isolates were included based on the CLSI guidelines (MIC) of imipenem and meropenem were determined using the agar dilution method and interpreted according to the CLSI guidelines. The results for the antimicrobial susceptibility test strain were interpreted as (S) susceptible, (I) intermediate, or (R) resistant by comparing the results to the CLSI 2017 [18] standard zone diameter Quality control strains used in antmicrobial susceptibility testing are Escherichia coli ATCC #25922 [18]. Minimum inhibitory concentration (MIC) of imipenem and meropenem were determined using the agar dilution method and interpreted according to the CLSI guidelines, the carbapenems resistant Enterobacteriaceae (CRE) isolates were included based on showing MICs ≥2 μg/mL for imipenem or meropenem were considered resistant. Only one isolate per patient was included [19].

2.5. Detection of Carbapenemase Blagenes

All 384 Enterobacteriaceae isolates were tested for 8 carbapenemase encoding blagenes by multiplex PCR. The carbapenemase gene was detected by PCR primers encompassing the entire coding region of genes blaKPC, blaGES, blaNDM, blaIMP, blaVIM, blaSIM-1, blaGIM-1 and blaOXA-48, according to [20] (MERK/Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany). Genomic DNA was extracted from overnight culture on LB (Luria-Bertani) media one single colony of each isolate by incubation in a final volume of 100 µL of distilled water at 95°C for 10 min followed by centrifugation [20]. Multiplex PCR was performed in a 20-µl reaction mixture; 2 µl of the extracted DNA, 0.5 µl from the primers, 10 µl of distilled water was added to 4µl of the PCR Master Mix (5x HOT FIREPol® Blend-Solis BioDyne, Estonia). Amplification was carried out with the following thermal cycling conditions by (Aeris Machine Pelter technology, Thermo Assist): initial denaturation step at 94°C of 10 min and 36 cycles of amplification consisting denaturation at 94°C of 30 s, annealing at 52°C of 40sec and extension at 72°C of 50sec, with 5 min at 72°C for the final extension following the protocol by [20]. The primers used for PCR amplification of the carbapenemase genes are listed in Table 1. PCR products were separated on a 2% agarose gel along with a 100bp DNA ladder (APSLABS, India) and then stained using safe green. The amplified products were then visualized with a transilluminator (Biometer an analytical Jena company).

2.6. Statistical Analysis

All calculations were performed using the Statistical Package for Social Sciences Statistics for Windows software package version 21.0 (SPSS-IBM, Armonk, NY). Results were presented using frequency and percentages for qualitative variables and as mean (standard deviation) for quantitative variables. Categorical variables were compared by Chi-square test and all tests were two-sided, and differences with P-value <0.05 were considered statistically significant. Odds ratio and 95% CI were estimated by multinomial logistic regression for risk of CPE infection.

2.7. Ethical Considerations

Ethical approval for this study was obtained from the Graduate College-UMST and the Federal Ministry of Health, Sudan-Research Ethics Committee (Reference number: 29.478-2018). Besides; the patients were informed about the study and the informed consent form was signed. Confidentiality was assured. No names were in the format used. The data were to be used for research only.

| PCR          | CPE blagenes | Oligonucleotide sequence (5′ → 3′)               | Size (bps) |
|--------------|--------------|------------------------------------------------|-----------|
| Multiplex I  | IMP          | F-TTGAACCTCCATTTTACDG                           | 139       |
|              |              | R-GATYGAAGATTAAAGCCACYCT                        |           |
|              | VIM          | F-GATGGTGTTTGGTCGATA                            | 390       |
|              |              | R-CGAATGCCGCAACCCAG                             |           |
|              | SIM-1 [21]   | F-TACAAGGGATTCGGCACG                            | 570       |
|              |              | R-AAATGCGCTGTCTCCCATGTG                        |           |
|              | GIM-1 [22]   | F-AGAACCTTGGACCCAGACG                            | 748       |
|              |              | R-ACTCATGACTCCTTCAGAGG                          |           |
| Multiplex II | OXA-48       | F-GCTTGATGGCCTCCTCGATT                         | 281       |
|              |              | R-GATTGGTCTCCGTGGCCGAAA                         |           |
|              | GES          | F-AGTCGCCGCTAGCCGGAAG                           | 399       |
|              |              | R-TTGTGCCGTGCCAGAT                             |           |
|              | KPC          | F-CATTCAAGGGCTTCTTGCG                           | 538       |
|              |              | R-ACGACGCCATAGCTTGTG                            |           |
|              | NDM          | F-GGTTTGCCGATTTGGTTC                           | 621       |
|              |              | R-CGGAATGGCTCATCAGATC                          |           |
3. Results

3.1. Demographic Characteristics of Patients with Enterobacteriaceae Infection

During 18 months, from a total of 1062 clinical samples collected, about 664 clinically significant isolates showed that 384 (36.2%) were Enterobacteriaceae, whereas the other 280 isolates included Gram-positive cocci (Enterococcus spp., and Staphylococcus spp.), Gram-negative bacilli non-glucose fermenter (Pseudomonas spp. and Acinetobacter spp.) and Gram-positive yeast (Candida spp.). Among the 384 Enterobacteriaceae isolates were isolated from arbitrarily of patients admitted in various hospital wards including medical wards (n=112; 29.2%), inflamed wound ward (n=93; 24.2%), respiratory ward (n=76; 19.8%), surgical ward (n=62; 16.1%), and renal ward (n=41; 10.7%). The mean age of patients 35.7 ± 14.3 years and 50.4% were female a slight preponderance versus 48.8% males (Table 2). The bacterial strains isolated included, Escherichia coli (n=144; 37.5%), Klebsiella pneumoniae (n=66; 17.2%), Enterobacter aerogenes (n=34; 8.9%), Citrobacter freundii (n=27; 7.0%), Enterobacter cloacae (n=22; 5.7%), Proteus mirabilis (n=21; 5.5%), Providencia stuartii (n=19; 4.9%), (n=17; 4.4%) each for Klebsiella. spp and Morganella morganii, Klebsiella oxytoca (n=8; 2.1%), Salmonella. spp (n=5; 1.3%) and Providencia. spp (n=4; 1.0%). The majority of isolates were predominantly obtained from wound purulent discharges as swab (n=123; 23.0%), urine samples (n=98; 25.5%), sputum (n=89; 23.2%) while other recovered from urine catheter (n=32, 8.3%), other body fluid (n=18; 4.7%), (n=10, 2.6%) for each blood cultures and stool samples and plural fluid (n=4; 1.0%). E. coli was the most common isolate from wards except for respiratory where K. pneumoniae was the most prevalent isolate. Metadata in Additional file 1.

3.2. CPE Prevalence

Among the 384 Enterobacteriaceae isolates, 122 (31.8%) were defined as presumable CPE based on the Ertapenem susceptibility testing results. Molecular analysis confirmed that these 122 isolates carried Carbapenemase-encoding blagenes. CPE prevalence across three Khartoum State Teaching Hospitals were significant in hospitals 43.0% (55/128) at hospital OTH, 19.5% (25/128) at hospital BTH, and not significantly different at hospital IMTH 32.8% (42/128). (Table 2). The proportion of CPE isolates was higher in inpatients age 20-60 years than in the other two age groups (OR = 1.09, 95% CI = 0.58–2.05), the patient's age was insignificantly related to CPE (p=0.848). Patients’ gender was not significantly associated with CPE presence (p = 0.269) (Table 2). Among the 122 CPE, E. coli was the predominant species 40 (32.3%), followed by 24(19.7%) K. pneumoniae, 14(11.5%) E. aerogenes, 9(7.4%) for each C. freundii and P. stuartii, 6 (4.9%) for each M. morganii, E. cloacae, and Klebsiella spp., 4(3.3%) P. mirabilis, 2 (1.6%) Salmonella spp. and 1 (0.8%) for each K. oxytoca and Providencia spp.

3.3. Antimicrobials Resistance Patterns in CPE and Non-CPE

Generally, the results for antimicrobial susceptibility testing for the 122 CPE and 262 non-CPE revealed that Enterobacteriaceae strains showed a high resistance rate to most of the commonly used antibiotics tested (Figure 1). Overall, ≥81% of all the CPE strains were resistant to carbapenems antibiotics, extended-spectrum cephalosporins, ampicillin, amoxyclave, and gentamicin. Resistance to quinolones ranges from 76.2% to 77.9%, followed by resistance to aminoglycoside ranges from 74.6% to 36.9%. Whilst 63.9% of strains were resistant to tetracycline and tobramycin, 43.4% resistant to tetracycline, and 17.2% resistant to colistin. Therefore, the resistance rates in non-CPE isolates to β-lactam antibiotics were up to 60.3%-77.9%, 51.9% for gentamicin, 43.5% for co-trimoxazole, 36.6% for each tetracycline and tobramycin, 29.8% for ciprofloxac, 23.7% for norfloxac, and 17.6% for nalidixic acid, for carbapenems range from 14.9%-1.9% and 5.7% of non-CPE resistance to colistin.

| Variables                     | CPE (n=122) | Non-CPE (n=262) | Odds Ratio (95% CI) | P-value |
|-------------------------------|-------------|-----------------|---------------------|---------|
| Gender                        |             |                 |                     |         |
| Female (n=195)                | 67          | 128             | 1.18 (0.88-1.59)    | 0.269   |
| Male (n=189)                  | 55          | 134             |                     |         |
| Age group (years)             |             |                 |                     |         |
| <20 (n=35)                    | 10          | 25              | 1.13 (0.37-4.25)    | 0.848   |
| ≥20–60 (n=331)                | 106         | 225             | 0.99 (0.36-2.75)    |         |
| ≥61(n=18)                     | 6           | 12              | 1.21 (0.42-3.48)    |         |
| Teaching Hospitals            |             |                 |                     |         |
| IMTH (n=128)                  | 42          | 86              | 1.07 (0.68-1.69)    | <0.01   |
| OTH (n=128)                   | 55          | 73              | 2.13 (1.36-3.32)    |         |
| BTH (n=128)                   | 25          | 103             | 0.39 (0.24-0.66)    |         |

Notes: 1. Carbapenemase producing Enterobacteriaceae, 2. Non-carbapenemase producing Enterobacteriaceae, 3. Odds Ratio, proportion of contribution to explain the outcome valuable, 4. 95% confidence interval for the odds ratio, 5. Level of statistics significance, a. Ibrahim Malik Teaching Hospital, b. Omdurman Teaching Hospital, c. Baheri Teaching Hospital.
3.4. Distribution of Carbapenemase Encoding Blagenes

Based on the PCR assays among the 122 CPE isolates, 84.4% (103/122) of CPE harbored one blagenes, and the blaIMP, blaNDM, and blaOXA-48 were predominant in 37 (30.3%), 28(23.0%), and 21 (17.2%) of isolates producing carbapenemase type respectively. Whilst blaGES, blaKPC, blaGIM-1 and blaVIM were less common in 6 (4.9%), 5(4.1%), 3 (2.5%) and 2 (1.6%) of CPE isolates. The blaSIM-1 gene was detected only in (0.8%) CPE isolates. The predominating genotype in most organism species was blaIMP except for M. morganii, Klebsiella spp., Salmonella spp., and Providencia spp. which also included blaOXA-48 except for K. oxytoca. In K. pneumoniae the predominating genotype was blaNDM, blaOXA-48, and blaKPC which were also commonly found in E.coli and E. aerogenes. With first new CPE distribution of blaGIM-1 in our region was produced by three strains of E.coli and one strain of E. cloacae, whereas one strain of K. pneumoniae produces blaSIM-1. (Figure 2).

Among 122 CPE isolates 19 (15.6%) carrying two or more blagenes, were consisted of coproducing for 4.1% blaNDM and blaOXA-48, 2.5% for each blaIMP and blaVIM positive and blaOXA-48 positive, 1.6% of blaSIM-1 gene was detected only in (0.8%) CPE isolates. The predominating genotype in most organism species was blaIMP except for M. morganii, Klebsiella spp., Salmonella spp., and Providencia spp. which also included blaOXA-48 except for K. oxytoca. In K. pneumoniae the predominating genotype was blaNDM, blaOXA-48, and blaKPC which were also commonly found in E.coli and E. aerogenes. With first new CPE distribution of blaGIM-1 in our region was produced by three strains of E.coli and one strain of E. cloacae, whereas one strain of K. pneumoniae produces blaSIM-1. (Figure 2).

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The predominant CPE regarding specimen were wound exude swab 40 (32.8%) CPE blagenes harboured were 37.5% of blaIMP, 20.0% of blaNDM, 15.0% of blaOXA-48, 7.5% of blaKPC, 5.0% of blaGES and 2.5% of blaGIM-1, followed by 33 (27.0%) sputum CPE blagenes were 27.3% of blaIMP, 24.2% of blaNDM, 15.2% of blaOXA-48, 3.0% for each of blaKPC, blaGES, blaGIM-1 and blaSIM-1, urine 22 (18.0%) CPE blagenes harboured were 31.8% of blaIMP, 22.7% of blaOXA-48, 13.6% of blaNDM, 4.5% for each of blaKPC, blaGES and blaGIM-1, 10 (8.2%) for each other body fluids and urine catheter, and smaller percentages ranging from 4(3.3%), 2 (1.6%) and 1 (0.8%) for blood culture, plural fluid and stool specimens most of CPE harboured genes were blaIMP, blaNDM, blaOXA-48 and blaVIM. There was no apparent correlation/relationship between specimen type and CPE genotype with insignificant p value 0.275.

Table 3. Frequency of carbapenemase co-producing Enterobacteriaceae blagenes per species

| Species                          | No of isolates (n=122) | bla genes present |
|----------------------------------|------------------------|-------------------|
| S. marcescens                    | 5 (4.1)                | NDM and OXA-48    |
| E. coli                          | 1 (0.8)                | NDM, GES, OXA-48, and IMP |
| E. coli                          | 1 (0.8)                | GES and IMP-1     |
| E. coli                          | 1 (0.8)                | IMP and OXA-48    |
| E. coli                          | 1 (0.8)                | VIM, OXA-48, and GES |
| M. morganii                      | 1 (0.8)                | NDM, OXA-48, and GES |

Table 4a. Imipenem MICs profile of 122 CPE isolates

| Carbapenemase blagenes          | MICs Imipenem μg/ml |
|----------------------------------|---------------------|
|                                 | n(%)<4 | n(%)<8 | n(%)<16 | n(%)<32 | n(%)<64 |
| blaVIM (n=2)                     | 0(0.0) | 0(0.0) | 0(0.0) | 2(100.0) | 0(0.0)  |
| blaKPC (n=5)                     | 0(0.0) | 0(0.0) | 0(0.0) | 1(20.0)  | 4(80.0) |
| blaKPC (n=5)                     | 0(0.0) | 0(0.0) | 0(0.0) | 1(20.0)  | 4(80.0) |
| blaKPC (n=21)                    | 3(14.3) | 2(9.5) | 4(19.1) | 7(31.8)  | 3(14.3) |
| blaGES (n=6)                     | 0(0.0) | 0(0.0) | 0(0.0) | 2(33.3)  | 2(33.3) |
| blaSIM (n=1)                     | 0(0.0) | 0(0.0) | 0(0.0) | 1(100.0) | 0(0.0)  |
| blaNDM (n=28)                    | 3(10.7) | 0(0.0) | 7(25.0) | 9(32.1)  | 9(32.1) |
| blaGIM (n=3)                     | 0(0.0) | 0(0.0) | 1(33.3) | 2(66.7)  | 0(0.0)  |
| blaNDM/KPC/OXA-48/GES (n=2)      | 0(0.0) | 0(0.0) | 0(0.0) | 2(100.0) | 0(0.0)  |
| blaNDM/GES/OXA-48/IMP (n=1)      | 0(0.0) | 0(0.0) | 0(0.0) | 1(100.0) | 0(0.0)  |
| blaNDM/OXA-48 (n=5)              | 3(60.0) | 2(40.0) | 0(0.0) | 0(0.0)   | 1(20.0) |
| blaKPC (n=5)                     | 0(0.0) | 0(0.0) | 0(0.0) | 1(100.0) | 0(0.0)  |
| blaOXA-48 (n=3)                   | 2(66.7) | 1(33.3) | 2(66.7) | 0(0.0)   | 0(0.0)  |
| blaVIM/OXA-48/GES (n=1)          | 0(0.0) | 0(0.0) | 0(0.0) | 1(100.0) | 0(0.0)  |
| blaGES/GIM (n=1)                 | 0(0.0) | 0(0.0) | 0(0.0) | 1(100.0) | 0(0.0)  |
| blaIMP (n=1)                     | 0(0.0) | 0(0.0) | 0(0.0) | 1(100.0) | 0(0.0)  |
| blaIMP/OXA-48 (n=1)              | 0(0.0) | 0(0.0) | 0(0.0) | 1(100.0) | 0(0.0)  |
| blaIMP/VIM (n=3)                 | 0(0.0) | 1(33.3) | 1(33.3) | 1(33.3)  | 0(0.0)  |
| Total CPE (n=122)                | 9(7.4) | 5(4.1) | 8(6.6) | 25(20.5) | 49(40.2) |

Table 4b. Meropenem MICs profile of 122 CPE isolates

| Carbapenemase blagenes          | MICs Meropenem μg/ml |
|----------------------------------|---------------------|
|                                 | n(%)<4 | n(%)<8 | n(%)<16 | n(%)<32 | n(%)<64 |
| blaVIM (n=2)                     | 0(0.0) | 0(0.0) | 0(0.0) | 2(100.0) | 0(0.0)  |
| blaKPC (n=5)                     | 0(0.0) | 1(20.0) | 3(8.1) | 9(24.3)  | 2(5.4)  |
| blaKPC (n=5)                     | 0(0.0) | 1(20.0) | 3(8.1) | 9(24.3)  | 2(5.4)  |
| blaKPC (n=5)                     | 0(0.0) | 1(20.0) | 3(8.1) | 9(24.3)  | 2(5.4)  |
| blaKPC (n=21)                    | 6(28.6) | 6(28.6) | 4(19.0) | 4(19.0)  | 2(10.0) |
| blaGES (n=6)                     | 4(80.0) | 4(80.0) | 4(80.0) | 4(80.0)  | 4(80.0) |
| blaSIM (n=1)                     | 0(0.0) | 0(0.0) | 0(0.0) | 2(33.3)  | 2(33.3) |
| blaNDM (n=28)                    | 8(6.6) | 8(6.6) | 8(6.6) | 8(6.6)   | 8(6.6)  |
| blaGIM (n=3)                     | 0(0.0) | 0(0.0) | 0(0.0) | 2(33.3)  | 2(33.3) |
| blaNDM/KPC/OXA-48/GES (n=2)      | 0(0.0) | 0(0.0) | 0(0.0) | 2(33.3)  | 2(33.3) |
| blaNDM/GES/OXA-48/IMP (n=1)      | 0(0.0) | 0(0.0) | 0(0.0) | 1(100.0) | 0(0.0)  |
| blaNDM/OXA-48 (n=5)              | 1(20.0) | 2(40.0) | 1(20.0) | 0(0.0)   | 0(0.0)  |
| blaKPC (n=5)                     | 1(20.0) | 2(40.0) | 1(20.0) | 0(0.0)   | 0(0.0)  |
| blaKPC (n=5)                     | 1(20.0) | 2(40.0) | 1(20.0) | 0(0.0)   | 0(0.0)  |
| blaKPC (n=5)                     | 1(20.0) | 2(40.0) | 1(20.0) | 0(0.0)   | 0(0.0)  |
| blaKPC (n=5)                     | 1(20.0) | 2(40.0) | 1(20.0) | 0(0.0)   | 0(0.0)  |
| Total CPE (n=122)                | 5(4.1) | 7(5.7) | 10(8.2) | 24(19.7) | 50(41.0) | 26(21.3) |
3.5. Distribution of Impenem and Meropenem MICs

In 122 CPE blagenes; Enterobacteriaceae strains possessing the blaKPC, blaIMP, blaNDM, blaVIM, blaOXA-48, and blaSIM-1 and blaGIM-1 genes had the highest carbapenem resistance with these strains having imipenem and meropenem MICs of ≥32 µg/mL. The strains carrying the combination of blaNDM and blaOXA-48 with 40.0% having meropenem MICs of 4µg/mL and imipenem <4µg/mL. The other resistance regarding the MICs of meropenem and imipenem resistance among strains possessing other blagenes represented in (Tables 4a and 4b).

4. Discussion

The emergence and spread of CPE is a significant threat to hospitalized patients worldwide, mainly due to the drought of therapeutic options for treating infections with MDR bacteria. In Sudan, interest in the dissemination of carbapenemase encoding blagenes among Gram-Negative non-glucose fermenter and Enterobacteriaceae isolates became recently evident in Khartoum State mainly in the hospitals [11,16,23]. In this study, we aimed to investigate the prevalence of carbapenemase blagenes and antimicrobials resistance pattern of Enterobacteriaceae isolates from hospitalized patients across three Khartoum State teaching hospitals, which serve the majority of the residence of the State as well as those who are referred from other states: IMTH, OTTH, and BTH are located in Khartoum locality, Omdurman locality, and Khartoum North locality, respectively.

This study reveals an Enterobacteriaceae strains prevalence of 36.2% among clinical isolates across three major Khartoum State teaching hospitals. The Enterobacteriaceae strains infection among hospitalized patients in our region is very high compared to 18.3, 16.9%, and 11.2% observed in Chad, Morocco, and Iran [12,24,25]. The majority of the clinical samples yielding the isolates used in this study isolated from female patients (50.4%) and most patients (86.2%) from either gender belonged to the age group of 20-60 years. Wound purulent discharges as swab (23.0%), urine (25.5%), and sputum (23.2%) formed the bulk of the samples that yielded cultures of Enterobacteriaceae strains. E. coli isolates were predominantly isolated from urine samples (48.0%), urine catheter (43.4% and wound swab (37.4%), whereas K. pneumoniae isolates were predominantly isolated from pleural fluid (50.0%) and sputum (38.2%).

The study reveals a CPE prevalence of 31.8% among clinical isolates across three major Khartoum State teaching hospitals. The CPE prevalence varies broadly between geographic areas. Our findings are lower than studies in South Africa 68% [26], in Sudan 67.8% [11], and in Egypt 62.7% [27], respectively. However comparable to those observed in other African countries, such as Sudan [16], Nigerian, Tanzania, Uganda [28] and Egypt [14] where a study reported a prevalence of 37.3%, 33.5%, 23.1%, 28.6% and 28.9% [29], respectively. Furthermore, our prevalence is also much higher than that obtained in studies from China [30], Germany [31], Morocco [12], north Israel [32], and Spain [33]. A possible explanation for such high CPE prevalence may be due to restricted use of antibiotics in these countries compared to Sudan and African context, where most drugs are available over the counter without prescription by a clinician or high selective pressure use of beta-lactam antibiotics proposed as first-line treatment for bacterial infections.

The most common CPE isolates are E. coli, K. pneumoniae, Enterobacter spp. and less predominant organisms are C. freundii, M. morgani, and Providencia spp, and P. mirabilis and most of these isolates were from wound exudate swab, urine samples, and sputum in agreement with previous findings in three studies in Sudan [11], Morocco [12], Egypt [14] and India [34]. Wound or abscess, Lower respiratory tract infection (LRTI) associated with ventilator, Urinary Tract Infection (UTI) are the most frequent bacterial infection worldwide in patients with nosocomial and community-acquired infections, and Enterobacteriaceae are generally the causal agent [10].

In our study, the most prevalent carbapenemase blagenes among 122 Enterobacteriaceae isolates are blaKPC, blaNDM, and blaOXA-48, whilst blaKPC and blaVIM less predominant. The blaKPC, blaNDM, blaOXA-48 are now considered endemic in many countries and are rapidly disseminating among different Enterobacteriaceae species [35]. Similar to our study, high proportions of blaIMP positive clinical isolates were reported in Studies in Sudan 50.6% [16] and Tanzania 21.6% [29]. A possibly explaining the observation of blaIMP genes in our strains may due to an increase in the use of imipenem antibiotics are present in the environment regarding previous studies [36,37]. Since then, blaOXA-48, blaNDM, blaKPC and blaGES variants observed in our study are in agreement with observations in our region by [38]. However; the blaVIM genes detected only in 2 CPE isolates in our study; these percentages consider low when compared with previous studies in Sudan [16,38,39].

We report the emergence of four blaGIM-1 producing Enterobacteriaceae strains also reported in a study from Germany [40]. Also, blaSIM-1 was detected in one strain; also reported recently in the study from China [41]. Suggesting that these isolates possessed the genes that may be due to mutation, horizontal gene transfer among other species, or an increase of international travel and patient transfer between countries to our hospital setting. We recommend further investigations to monitor CPE among both hospital and community-based studies besides to determine CPE carrier in our community healthy individuals [35].

Interestingly, 15.6% of CPE in this study were found to coproducing of carbapenemase encoding blagenes, a combination of carbapenemase genes blaNDM and blaOXA-48(4.1%) detected among 3 of E.coli and one for each K.pneumoniae and M. morgani isolates, also the same result were reported in South Africa [26] and Gulf area [42]. To the utmost of our knowledge, this is the first report of the coproducing of 4 carbapenemase gene types of blaNDM, blaKPC, blaOXA-48, and blaGES positive, blaNDM, blaGES, blaOXA-48 and blaIMP positive and blaGES and blaGIM-1 positive in E.coli and K.pneumoniae at Khartoum state area of Sudan. Also 2.5% for each combination blaOXA-48+blaGES and
In this study, all CPE isolates were highly resistant to all 3rd generation cephalosporins, β-lactam without/with inhibitor, aztreonam, carbapenems, quinolones, and aminoglycosides except (amikacin) more than non-CPE isolates also reported in [16,26,39,43]. Comparing the results of the present study with these earlier studies indicates that the resistance rate of CPE to various antibiotics has not decreased either in our region or in African nearby countries. The being of >80% susceptibility of CPE isolates to colistin in this study indicated that polymyxins is still a better option of the drug for the treatment of infections caused by CPE in Khartoum state hospitals and reported in studies [11,16,39]. However, colistin had the issue of nephrotoxicity, neurotoxicity [44]; regarding the spreading of CPE encoding genes was became emergent in Khartoum state hospitals, the colistin-resistance increased 17.2% as no longer efficacious in the treatment and management. Therefore an urgent need to address a direct impact on control practices of antibiotics misuse and establishing antimicrobial stewardship programs.

5. Conclusion

These studies will contribute to better understand the distribution of CPE in hospitals, CPE prevalence (31.8%), and the predominance of the blaIMP, blaNDM, and blaOXA-48 among clinical isolates across three Khartoum State teaching hospitals, besides emphasizes the first detection of blaGIM-1 and blaSIM-1 in our region. Further investigations to monitor CPE in regional and national–level surveillance programs may require tracking the evolution and spread of CPE mediated antimicrobials resistance. Further studies may be performed to characterize the novel types in the future.

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Conflicts of Interest

Authors declare no conflict of interest.

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Data Availability

All data generated or analyzed during this study are included in this published article (are available from the corresponding author on reasonable request as (Excel file1).

Author Information

OMH, Conceived, designed and performed the experiments. OMH writing of the manuscript and MAHB manuscript revised and supervised the research work. OMO for sample collection during the data collection period. All authors read and approved the final manuscript.

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