

The Emergence of Carbapenem Resistant *Enterobacteriaceae* Producing GIM-1 and SIM-1 Clinical Isolates in Khartoum-Sudan

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Purpose: The aim of this study was to detect multidrug resistant GIM-1 and SIM-1 producing *Enterobacteriaceae* clinical isolates from hospitalized patients across three Khartoum State Teaching Hospitals, Sudan.

Patients and Methods: From May 2018 to October 2019, *Enterobacteriaceae* clinical isolates from inpatients admitted to different Khartoum state hospitals. Genes for carbapenemase (GIM-1 and SIM-1) were amplified by polymerase chain reaction (PCR). Agar dilution method was used to determine MICs for imipenem and meropenem after antimicrobial susceptibility testing.

Results: Five (1.29%) isolates of *Enterobacteriaceae* [2 (0.51%) *Escherichia coli* isolates produce GIM-1, 2 (0.51%) *Klebsiella pneumoniae* isolates (one [0.25%] of each produce of GIM-1 and of SIM-1), and 1 (0.25%) *Enterobacter cloacae* isolate produce GIM-1]. Susceptibility profiling of the isolates showed a low-level resistance to imipenem and meropenem MICs (8, 16 and 32 µg/mL). It also had resistance to ampicillin, extended-spectrum cephalosporin's, aztreonam, and amoxicillin-clavulanate and with the two *K. pneumoniae* strains showing resistance to colistin.

Conclusion: We report the emergence of four GIM-1 producing *Enterobacteriaceae* strains and one strain of SIM-1 producing *K. pneumoniae* genes, isolated from hospitalized patients, with a high resistance pattern to antimicrobial agents. Whole-genome sequencing (WGS) is necessary for precise identification of clonal diversity backgrounds of acquired carbapenemase genes in diagnostic laboratories as the number of cases of carbapenem resistant *Enterobacteriaceae* infection increases annually.

Keywords: *Enterobacteriaceae*, metallo-β-lactamase (MBL), GIM-1 and SIM-1 genes

Introduction

There is a lot of concern regarding Gram negative strains because of the high number of hospital- and community-acquired infections, in addition to the easy acquisition of resistance genes within the bacterial species.¹ The emergence of newly acquired carbapenemase genes amongst *Enterobacteriaceae* isolates occurs predominantly in healthcare institutions, and is due to the presence of exogenous genetic materials such as plasmids, transposons and insertion sequence common region (ISCR) elements containing genes coding for carbapenemase production.² Numerous families of acquired Metallo-β-lactamase (MBLs) have been identified, including IMP, VIM and NDM, which are the most commonly acquired MBLs reported worldwide in the clinically important Gram-negative bacteria.³ Moreover, SPM, GIM, SIM, DIM, KHM, TMB, FIM and AIM are sporadic acquired MBLs in certain bacterial species and have a limited geographical distribution.⁴ VIM-, IMP- and NDM-type enzymes are the most commonly acquired MBLs in our region reported.⁵ Both German imipenemase (GIM-1) and Seoul imipenemase (SIM-1) are Ambler class B (MBL) mediated by acquired carbapenemase genes.^{6,7} However, GIM and SIM enzymes are being incorporated in a gene cassette inserted into a class 1 Integron, which plays a major role in the dissemination of β-lactamase genes and aminoglycoside modifying resistance genes.⁴ The first isolation of GIM-1 was reported for *Pseudomonas aeruginosa* isolates in Germany in 2002⁸ and has similar characteristics to those of the other acquired MBL, approximately 30% homology

to VIM, 43% homology to IMPs, and 29% homology to SPM. The GIM-1 gene was also detected in *P. putida*, *E. cloacae*, *S. marcescens*, *E. coli*, *K. oxytoca*, *Citrobacter freundii*.⁹ The enzyme SIM-1 has the closest amino acid identity to the IMP family (64% to 69%) and the first reported from Korea in imipenem resistant *Acinetobacter baumannii* isolates¹⁰ and in two other strains *A. pittii* and *A. nosocomialis*.¹¹ A thorough understanding of the bacterial species involved and their antibiotic resistance patterns in a particular geographical area is essential, to adequately treat these patients. There are few data available on the emergence of newly acquired carbapenemase genes among *Enterobacteriaceae* in our region. The aim of this study is to detect multidrug resistant *GIM-1* and *SIM-1* producing *Enterobacteriaceae* clinical isolates from hospitalized patients across three Khartoum State Teaching Hospitals, Sudan.

Materials and Methods

Enterobacteriaceae Clinical Isolates

This clinical study was carried out at UMST and three major Khartoum state Hospitals, Sudan, from May 2018 to October 2019. Total of 385 non-duplicative clinical isolates of *Enterobacteriaceae* were obtained from different clinical samples, including urine (n = 130), wound swabs (n = 123), sputum (n = 89), Other Body Fluid (n = 18), blood (n = 10), stool (n = 10), and Plural Fluid (n = 4). The isolates were identified based on their colony characteristics, Gram stain, and a panel of gram-negative biochemical set and unidentified strain confirmed by API 20E (bioMérieux, France). Additionally, the direct interviewing questionnaires were filled out to obtain demographic data such as the age, gender, date of hospital admission, ward, comorbidities, and recent travel history of the participants in order to evaluate risk factors for *GIM-1* and *SIM-1* producing *Enterobacteriaceae*. Each participant agreed to be included in this study voluntarily, so the consent form was signed before collecting the clinical samples and questionnaires. A study ethics approval was obtained from UMST, Khartoum, Sudan, 2017, and the Ethical Committee of the Ministry of Health of the Sudan (FMOH-Human) (reference no. 29.478/2017).

Antimicrobial Susceptibility Testing

This was performed with the disk diffusion method (Kirby-Bauer's) and the inhibition zone diameters were measured by (mm) according to Clinical and Laboratory Standards Institute (CLSI).¹² The antimicrobial discs tested include Amoxicillin/clavulanic acid (30 µg), ampicillin (10 µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), aztreonam (30 µg), imipenem (10 µg), meropenem (10 µg), ertapenem (10 µg), gentamicin (10 µg), tobramycin (10 µg), amikacin (30 µg), tetracycline (30 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), nalidixic acid (30 µg), co-trimoxazole (25 µg) and colistin (10 µg) 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 2018 standard zone diameter.¹² Minimum inhibitory concentrations (MICs) for imipenem and meropenem were performed by the agar dilution method and interpreted according to CLSI guidelines (strains displaying MICs ≥8 µg/mL for imipenem and meropenem were considered resistant).¹² *Escherichia coli* ATCC 25922 was used as standard control strains.

Detection of *GIM-1* and *SIM-1* Genes

For preparation of deoxyribonucleic acid (DNA), the template of the investigated *Enterobacteriaceae* clinical isolates were extracted by boiling technique as follows: four colonies of each strain were emulsified in 200 µL sterile deionized water and then heated at 95°C for 30 min and frozen at -20°C for 30 min. After thawing, the tube centrifuged at 14,000 rpm for 10 min. The supernatant was then aliquoted and preserved at -20°C for future use.¹³ The extracted DNA from *Enterobacteriaceae* isolates was used for PCR amplification of genes primers sets of *GIM-1*⁸ (F-AGAACCTTGACCGAACGCAG, R-ATCATGACTCCTCAGAGG-748bp) and *SIM-1*¹⁴ (F-TACAAGGGATTCGGCATCG, R-TAATGGCCTGTTCCCA TG TG-570bp). All PCR-reaction conditions were prepared by using 1 µL of extracted DNA template, 20 pmol of each primer, and 4 µL of 5x HOT FIREPol® Blend Master Mix Ready to Load (Solis BioDyne, Tartu, Estonia) containing 1 U of Taq DNA polymerase in a total volume of 20 µL by Peltier effect thermal cycler (Aeris™) was used with the following reaction conditions: one cycle 95°C for 5 min (initial denaturation), 30 cycles of 94°C for 40 s, 52°C for 1 min, and 72°C /2 min, and finally, 72°C for

5 min. The amplified PCR products were analysed using 1.5% agarose gel electrophoresis along with 100 bp ladder DNA. We stained the gels with ethidium bromide and visualized them using a UV transilluminator system (Biometer, an analytical company in Jena).

Statistical Analyses

The Statistical Package for Social Sciences Statistics for Windows software package version 21.0 (IBM, Armonk, NY) was performed for data analyses. Results were expressed using frequency and percentages.

Results

Demographic and Clinical Characteristics

A total of 385 (36.25%) isolates of *Enterobacteriaceae* were isolated from clinical specimens of 1064 patients. Since the study focuses on the detection of GIM-1 and SIM-1 of *Enterobacteriaceae* clinical isolates, samples positive for the organism were collected for further characterization. The frequency of GIM-1 producing *Enterobacteriaceae* strains was (n=4; 1.04%), two strains of *K. pneumoniae* (n = 1; 0.26%) and *E. cloacae* (n = 1; 0.26%) were recovered from sputum culture of patients diagnosed with Lower respiratory tract infection (LRTI); 22 years old male LRTI-associated with ventilator and 64 years old female associated with breast abscess, respectively, and both patients had no travel history. GIM-1 was also detected in *E. coli* strains (n = 2; 0.52%); one strain was isolated from wound swabs of a 21-year-old woman with postoperative wound infection, and another strain was isolated from a urine sample of a 33-year-old man with renal disease comorbidity associated with urinary tract infection. These two patients had previously travelled to nearby countries. The SIM-1 gene was produced by *K. pneumoniae* (n = 1; 0.26%) isolated from sputum sample of a female patient aged 24 with a lower respiratory tract infection related to visceral leishmaniasis, who had previously travelled.

Antimicrobial Susceptibility Testing

The five strains of GIM-1 and SIM-1 showed 100% (n = 5) resistant to extended-spectrum cephalosporins, carbapenems, gentamicin and amoxicillin/clavulanic acid antibiotics and 20% (n = 1) resistant to the non- β -lactam antibiotics, ciprofloxacin, nalidixic acid and colistin. Both strains of *K. pneumoniae* isolates shows susceptible to only one antimicrobial and categories as extensively drug-resistant (XDR) shown in (Table 1).

Genotypic Detection of GIM-1 and SIM-1

Consequent multiplex PCR analysis revealed that four isolates each of 0.52% of *E. coli* (n = 2), 0.26% of *K. pneumoniae* (n = 1) and 0.26% of *E. cloacae* (n = 1) carried GIM-1 like and 0.26% (n = 1) of *K. pneumoniae* harboured SIM-1 like. The strains possessing the GIM-1 gene had the highest carbapenem resistance with these strains having imipenem and meropenem MICs of 32 μ g/mL or more. This was followed by the strains carrying the SIM-1 gene having imipenem MICs of 8 μ g/mL or more, and having meropenem MICs of 16 μ g/mL or more shown in (Table 1).

Discussion

Increasingly, carbapenem-resistant gram-negative bacteria have been detected worldwide as well as in Sudan.^{5,15–17} Here we report the presence of GIM-1 and SIM-1 producing *Enterobacteriaceae* clinical isolates in Sudan. Effective and accurate screening of CPE strains with high resistance to carbapenem is important in routine clinical microbiology laboratory. In 2002, GIM-1 was first identified in *P. aeruginosa*,⁴ and later has been identified in 50 isolates of *P. aeruginosa*, *E. cloacae*, *P. putida*, *S. marcescens*, *E. coli*, *K. oxytoca* and *C. freundii* from one hospital outbreak in Italy 2007–2012.⁹ Further studies in Germany were also reported the GIM-1 producing *S. marcescens* and *E. cloacae* in 2012¹⁸ and 2013,¹⁹ respectively. Recently GIM-1 harbouring *A. baumannii* and *P. aeruginosa* were reported for the first time in Egypt^{20,21} respectively. The GIM-1 gene is no longer associated only with Germany's geographical region, as previously thought, as recently ongoing to spread and report in other world regions is common. Our data presented the 1.56% of clinical isolates of *Enterobacteriaceae* were GIM-1-producing (*E. coli*, *K. pneumoniae* and *E. cloacae*) with

Table 1 Demographic Characteristics, Antimicrobial Susceptibility Profile, Carbapenem MICs of the GIM-I- and SIM-I Producing *Enterobacteriaceae* Isolates

Carbapenemase Producing <i>Enterobacteriaceae</i> bla _{genes}	<i>K. pneumoniae</i> SIM-I	<i>K. pneumoniae</i> GIM-I	<i>E. coli</i> GIM-I	<i>E. coli</i> GIM-I	<i>E. cloacae</i> GIM-I
Location of Patient and Hospital Admission/Day	Respiratory Ward /7 Days	Respiratory Ward/>7 Days	Medicine Ward/<7 Days	Renal Ward/ >7 Days	Respiratory Ward/7>Days
Specimen/Diagnosis	Sputum/LRTI	Sputum/LRTI	W/S-POWI	Urine/UTI	Sputum/LRTI
Antimicrobial susceptibility pattern (mm)					
Amoxicillin/clavulanic acid	R	R	R	R	R
Ampicillin	R	R	I	S	I
Cefotaxime	R	R	R	R	R
Ceftazidime	R	R	R	R	R
Ceftriaxone	R	R	R	R	R
Aztreonam	I	R	R	R	R
Imipenem	R	R	R	R	R
Meropenem	R	R	R	R	R
Ertapenem	R	R	R	R	R
Gentamicin	R	R	R	R	R
Tobramycin	R	R	S	S	S
Amikacin	R	S	S	R	S
Tetracycline	R	R	S	R	S
Ciprofloxacin	R	I	I	I	S
Norfloxacin	R	I	R	R	I
Nalidixic acid: NA	R	I	I	S	I
Co-trimoxazole	R	R	R	S	S
Colistin	I	R	S	S	S
MIC test (µg/mL)					
Imipenem	8	32	32	16	16
Meropenem	16	32	32	32	32

Note: Inhibition zone by mm according CLSI-2018.

Abbreviations: W/S, wound swab; POWI, postoperative wound infection; LRTI, lower respiratory tract infection; UTI, urinary tract infection; MIC, minimum inhibitory concentration; R, resistant; I, intermediate; S, susceptible.

a high resistance pattern to cephalosporins, carbapenems, gentamicin, and Amoxicillin/clavulanic acid and low resistance to other antimicrobial classes, suggesting that the enzyme may contribute to broad-spectrum resistance. It is of interest that SIM-1 was produced by one strain of MDR *K. pneumoniae* intermediately susceptible to aztreonam and colistin. Similar results were reported for the SIM-1 gene in *A. baumannii*¹⁰ in Iraq²² and in Egypt,²⁰ in *P. aeruginosa* and recently in Egypt²³ and *K. pneumoniae* with ST23 strain carrying SIM-1 in China.²⁴ The GIM-1 and SIM-1 genes were transferred to Sudan, these can be broadly categorized into issues of emergence, spread, and clonal expansion with diversity, which include natural genetic diversification caused by mutation, horizontal gene transfer among other species, an increase in international travel and patient transfer between countries raising the possibility for further dissemination of strains carrying resistant genes. There is a need for further investigation regarding travel history, as three of the patients had previously travelled.

Conclusion

A newly acquired carbapenemase, GIM-1 and SIM-1, is detected in five clinical isolates of *Enterobacteriaceae* from Sudan. Four strains produce GIM-1, while a single strain of *K. pneumoniae* produces SIM-1, with limited susceptibility

to antimicrobial agents. Additionally, whole-genome sequencing (WGS) is necessary to assess the genetic mapping and sequence types of the GIM-1 and SIM-1 integron structures in our region.

Data Sharing Statement

All data used and/or evaluated during this study are included in this published article (are available from the corresponding author on reasonable request).

Ethics Approval

We would like to confirm that our study complies with the declaration of Helsinki. Besides ethical approval for this study was obtained from the Ethical Committee of the Sudan Federal Ministry of Health (FMOH-Human) (reference number: 29.478/2017), and from Graduate College – University of Medical Sciences and Technology UMST, Khartoum, Sudan, 2017. The purpose of the study was explained in detail to all participants or their relatives before a written and verbal consent form was signed on a voluntary basis.

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Disclosure

The authors declare that they have no conflicts of interest in this work.

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