Scientific Research Publishing

American Journal of Molecular Biology, 2022, 12, 85-96
https://www.scirp.org/journal/ajmb
ISSN Online: 2161-6663
ISSN Print: 2161-6620

Incidence of Oxa23 and Oxa51 Genes Associated with Bacterial Isolated from Patients with Urosepsis: Single Centre Prespective

Salma Omer Ibrahim[1*], Elimam M. A. Mohammed[2], Sami Mahjoub Taha[2], Sanaa Mohammed Yousif[3], Hajir Omer[1], Omer Omer[4], Mirghani Seif-Elnasr[5], Seitelbanat Yassin[6], Yousif Abdelhameed Mohammed[7], Omer Abu Elhasan[8], Mohamed Taj-Eldin[8], Adam Dawoud Abakar[9], Elhadi Abdalla Ahmed[1], Salaheldein G. Elzaki[10]

[1]Department of Medical Microbiology, Faculty of Medical Lab Sciences, University of Gezira, Wad Medani, Sudan
[2]Department of Urology, Faculty of Medicine, University of Gezira, Wad Medani, Sudan
[3]National Blood Bank, Ministry of Health, Khartoum, Sudan
[4]University Hospital Galway, Galway, Ireland
[5]Department of Urology, Gezira Hospital for Renal Disease and Surgery, Wad Medani, Sudan
[6]Department of Pharmaceutics, Faculty of Pharmacy, University of Gezira, Wad Medani, Sudan
[7]Department of Clinical Chemistry, National Cancer Institute, University of Gezira, Wad Medani, Sudan
[8]Department of Medical Microbiology, Wad Medani College for Medical Science and Technology, Wad Medani, Sudan
[9]Department of Medical Parasitology, Faculty of Medical Laboratory Sciences, University of Gezira, Wad Medani, Sudan
[10]Department of Epidemiology, Tropical Medicine Research Institute, Khartoum, Sudan
Email: *salooma.omer@gmail.com

How to cite this paper: Ibrahim, S.O., Mohammed, E.M.A., Taha, S.M., Yousif, S.M., Omer, H., Omer, O., Seif-Elnasr, M., Yassin, S., Mohammed, Y.A., Elhasan, O.A., Taj-Eldin, M., Abakar, A.D., Ahmed, E.A. and Elzaki, S.G. (2022) Incidence of Oxa23 and Oxa51 Genes Associated with Bacterial Isolated from Patients with Urosepsis: Single Centre Prespective. *American Journal of Molecular Biology*, **12**, 85-96.
https://doi.org/10.4236/ajmb.2022.123008

Received: March 31, 2022
Accepted: May 28, 2022
Published: May 31, 2022

Abstract

Background: Urosepsis is one of the most common infections that require empirical broad spectrum antibiotics immediately after diagnosis. This has led to development of bacterial resistance by acquiring the capability to destroy the β-lactam ring. **Methodology:** This is a cross-sectional hospital-based study. The study was conducted from 2019 to 2020 at Gezira Hospital for Renal diseases and surgery (GHRDS). A hundred patients were diagnosed clinically with urosepsis and the isolated organisms were *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*. The susceptibility test was conducted by Kirby Bauer disc diffusion technique according to clinical laboratory standard institute (CLSI) guidelines. Seventy eight samples of bacterial genomic DNA were confirmed by 16srRNA and multiplex PCR, were performed for genotypic blaOXA-51 and blaOXA-23 gene characterization of isolated bacteria. Then gel electrophoresis was used to identify the presence or absence of (blaOXA-51 and blaOXA-23) genes. **Results:** 88.5% (69/78) in 16srRNA detected. Using multiplex PCR,

the frequencies of blaOXA-51 and blaOXA-23 genes were 13% and 10.1%, respectively. The percentages of isolates which yielded both blaOXA-51 and blaOXA-23 among *P. aeruginosa* was 25% (1/4), among *K. pneumonia* was 17% (1/6), and among *E. coli* was 8% (3/37). Only blaOXA-51 was detected in *P. mirabilis* 10% (1/10) and only blaOXA-23 was detected in *S. aureus* 5% (1/18). **Conclusion:** In this study, the presence of blaOXA-51 and blaOXA-23 genes was increased in the isolated bacteria.

Keywords

Urosepsis, Carbapenem-Resistant Enterobacteriaceae (CRE), blaOXA-51 and blaOXA-23

1. Introduction

Urosepsis is defined as a life-threatening condition resulting from a dysregulated response to infections of male genital tract or urinary tract (UT). The complication of urosepsis can lead to multiple organ dysfunction syndrome and death. Nearly 20 - 30 percent of sepsis cases originated from the urogenital tract [1] [2].

The most common isolated pathogens that cause urosepsis are *Escherichia coli*, *followed by Proteus mirabilis*, *Enterobacter*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and gram-positive bacteria [3] [4] [5]. These causative organisms are considered serious invaders which need urgent treatment and are classified into critical (including; *P. aeruginosa*, *E. coli*, *P. mirabilis*, *K. pneumonia*) and high (including; *S. aureus*) according to World Health Organization (WHO) [6]. The customary empirical usage of late-generation of cephalosporins yields unlimited development of bacterial resistance [7]. The majority of these strains acquired the capability to destroy the β-lactam ring [8] [9] [10] in cephalosporins. Therefore carbapenems were used as an effective alternative to the broad-spectrum antibiotic to treat these bacteria. However, organisms express resistance to carbapenems [11].

Globally; in the last decade strains that revealed resistance to carbapenemfell mainly in gram negative bacteria including; *Acinetobacter baumannii* and *Pseudomonas aeruginosa* by different mechanisms intrinsic or mediated by transferable carbapenemase-encoding genes [12] these mechanism includes; decreased membrane permeability, efflux pumps and an enzymatic resistance to carbapenems. Carbapenemase is enzymatically classified into Ambler class A, B and D based on amino acid homology [13] [14] [15]. Acquired class D β-lactamases, also known as oxacillin hydrolyzing enzymes (OXA) genes, structurally are different in amino acid from class A and C enzymes and are widely distributed among gram negatives rods. This resistance is mostly associated with class 1 integron or insertion sequences. These genes are characterized by important genetic diversity and great heterogeneity in terms of β-lactam hydrolysis spectrum [16]. This class contains various types of carbapenemase and generally cannot be deactivated by clavu-

lanic acid, tazobactam, and sulbactam and it is including blaOXA-51, blaOXA-23, blaOXA-24/40, blaOXA-58, blaOXA-143, and blaOXA-235. Most of them are encoded by chromosomal genes with OXA-48 considered the most commonly detected [15] [17] [18].

The recent acquisition and encoding of carbapenem-resistance by chromosomal genes (OXA-51), plasmid-mediated genes (OXA-23) [11] [19] [20], and production of cephalosporinases combined with mutations associated with decreased permeability of the bacterial cell wall. These enzymes have the capability to hydrolyze and inactivate beta lactam rings are found in beta lactam antimicrobials [17] [21], which led to emergence of carbapenem-resistant enterobacteriaceae (CRE) [22] [23].

Carbapenem resistance (CR) is globally distributed in most bacteria [24] [25]. In Sudan many studies dealt with extended-spectrum beta-lactamases (ESBL) genes and carbapenem resistant [26] [27]. This study aims to determine the incidence of oxa23 and oxa51 among isolated bacteria from urosepsis patients admitted to Gezira Hospital for Renal Diseases and Surgery, Sudan (GHRDS).

2. Methodology

2.1. Patients and Methods

This is a cross-sectional hospital-based study. It was conducted from 2019-2020 at (GHRDS), Wad Medani, Sudan. GHRDS is considered the only specialized referred centre outside Khartoum. A hundred samples were selected from patients diagnosed clinically in urology department with urosepsis caused by drug-resistant pathogens. The participants were in different ages and genders and came from different States in Sudan.

2.2. Identification of Bacterial Isolates and Susceptibility Testing

Urine samples were collected in sterile urine containers then inoculated on cysteine lactose electrolyte deficient (CLED) media, overnight at 37˚C, then identified by colonial morphology, lactose fermentation and gram's stain. Further biochemical tests identified the bacterial isolates and the antimicrobial susceptibility testing was performed by Kirby Bauer disc diffusion technique for selected antimicrobial agent according to clinical and laboratory standards institute (CLSI). The most common β-lactam antibiotics prescriptions in policy of urology department of GHRDS are third and fourth cephalosporin (ceftriaxone, ceftizoxime and cefepime) and carbapenem (meropenem).

2.3. Molecular Identification

2.3.1. Bacterial Genomic DNA Extraction

Extracted genomic DNA fromisolated bacteriain different antimicrobial susceptibility pattern were performed using (G-spin TM Total) iNtron (South Korea, Soul), Lot. No. 105251551. The extracted DNA was stored at −80˚C till PCR analysis.

2.3.2. 16srRNA Amplification

From extract used Primers for 16srRNA amplification, blaOXA-51 and blaOXA-23 showed in Table 1. Measurement of DNA concentration and purity quality was accomplished with a NanoDrop spectrophotometer (Bibby Scientific, UK).

2.3.3. PCR Protocol Used for Multiplex to Detect blaOXA-51, blaOXA-23

In this study, we performed two molecular tests. A single PCR reaction for the detection of bacterial 16srRNA gene and a multiple exprotocol for detection of OXA-23 and OXA-51 genes In the protocol of 16srRNA, the initial denaturation was done at 95˚C for 6 minutes for initial denaturation followed by 40 cycles at 95˚C for 30 seconds for denaturation, 50˚C for 50 seconds for annealing, and 72˚C for 60 seconds for extension. The final extension was 10 minutes at 72˚C. In the protocol of multiplex PCR, an initial denaturation at 94˚C for 3 minutes is followed by 35 cycles of 94˚C for 45 seconds, 57˚C for 45 seconds, and 72˚C for 1 60 seconds, with a final extension for 5 minutes at 72˚C.

The PCR product processed in 1% agarose gel electrophoresis was performed to identify the presence or absence of (blaOXA-51 and blaOXA-23) genes.

3. Ethical Approval

The ethical approval was obtained from GHRDS, Faculty of Medical Laboratory Sciences, University of Gezira and Ministry of Health, Gezira State, Sudan.

4. Results

From 100 patients diagnosed clinically as urosepsis in the urology department of GHRDS during the study period, the majority of samples were males from different states with a median age of 45years. The result was shown in (Table 2). Urine samples were cultured on CLED aerobically along with urinalysis. *E. coli* was the predominant bacteria with a high frequency of isolation (Figure 1). Antimicrobial susceptibility test (AST) showed most of the isolates were prevalent with complete resistance to cefepime as shown in (Table 3). The identities of 88.50% (69/78) of isolates were confirmed with 16srRNA. The length of bands was approximately 1500 bp as shown in (Figure 2). 16srRNA of *E. coli* was detected at 89.2% (33/37), with that of *S. aureus* at 85.7% (18/21), *P. mirabilis* at 10/10 (100%), *K. pneumonia* at 83.3% (5/6), and *P. aeruginosa* at 25% (1/4) as

Table 1. Primers use for amplification of 16srRNA, oxa23 and oxa51 in this study.

Primer	Sequence	Fragment size	
16srRNAF 16s RNA R	5'-AGAGTTTGATCCTGGCTCAG-3' 5'-GGTTACCTTGTTACGACTT-3'	1500	[28]
Oxa23 F Oxa23 R	5-GAT CGG ATT GGAGAACCA GA-3 5-ATT TCTGACCGC ATT TCC AT-3	501 bp	[29]
Oxa51 F Oxa51 R	5-TAATGCTTT GAT CGGCCT TG-3 5-TGG ATT GCACTT CAT CTTGG-3	353 bp	[29]

Table 2. Social demographic data of urosepsispatients in GHRDS 2019-2020.

		Patients (n)	Percent
Gender	Male	70	70
	Female	30	30
	Total	100	100
Age	1 - 16 Years	7	7
	17 - 40 Years	18	18
	41 - 60 Years	31	31
	>61 Years	44	44
	Total	100	100
State	Gezira	69	69
	Sinnar	11	11
	Elqadarif	7	7
	Eldamazin	10	10
	Others	3	3
	Total	100	100

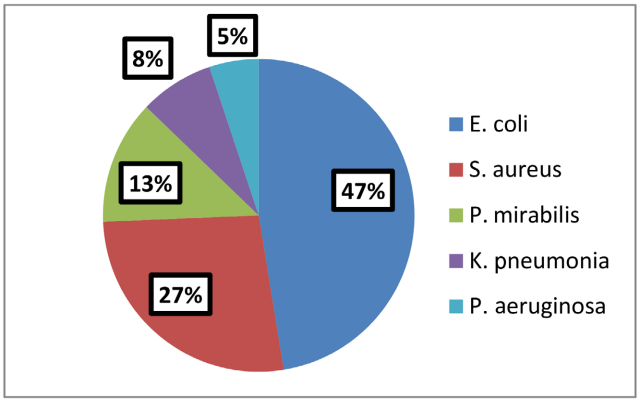

Figure 1. The incidence of the bacterial isolates from urosepsis patients.

Table 3. The results of antimicrobial susceptibility/intermediate-resistant/resistant of isolated strains.

	Sensitive		Intermediate		Resistant	
	F	%	F	%	F	%
Meropenem	70	90	0	0	8	10
Ceftizoxime	42	54	9	12	27	35
Ceftriaxone	11	14	7	9	60	77
Cefepime	0	0	0	0	78	100

showed in **Table 4**. Obtained bands by multiplex PCR for OXA-23 and OXA-51 were 501 bp and 353 bp, respectively (**Figure 3**). The frequency of blaOXA-23 and blaOXA-51 gene 10.1% and 13%, respectively showed in **Table 5**. Presence of

Table 4. Detected DNA of isolated bacteria by 16srRNA.

Isolated Microorganism	No Product		Detect		Total
	F	%	F	%	
E. coli	4	11	33	89	37
S. aureus	3	14	18	86	21
P. mirabilis	0	0	10	100	10
K. pneumonia	1	17	5	83	6
P. aeruginosa	3	75	1	25	4
Total	11	14	67	86	78

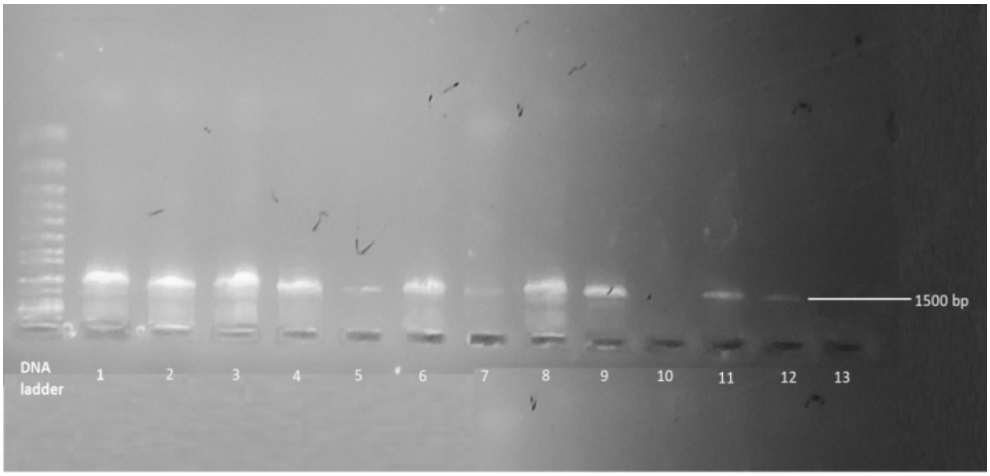

Figure 2. PCR amplification of 16srRNA.

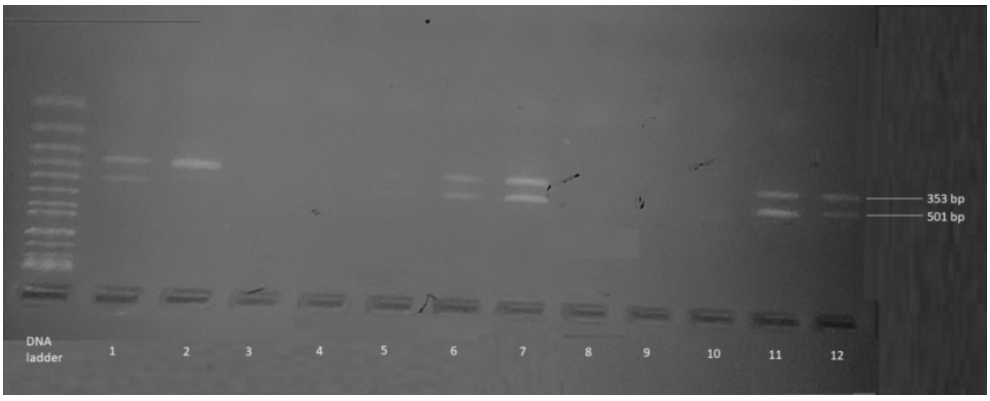

Figure 3. Differentiation of oxa23 (501 bp) and oxa51 (353 bp).

Table 5. The incidence of blaOXA-51 and blaOXA-23.

	Oxa51		Oxa23	
	Frequency	Percent	Frequency	Percent
Absent	60	87	62	89.9
Present	9	13	7	10.1
Total	69	100	69	100

blaOXA-51 and blaOXA-23 among isolated resistant strain associated with result of susceptibilty, cefepime showed full resistance, furthermore; meropenem and ceftizoxime showed significant p value 0.000, shown in Table 6 & Table 7. Eight percent (3/37) and 14% (3/18) of *E. coli* and S. aureus isolates possessed blaOXA-51 gene. While 25% (1/4) of *P. aeruginosa* showed blaOXA-51 and blaOXA-23 genes (Table 8).

Table 6. The correlation between the presence of oxa23 and antibiotics resistance.

Antibiotics	Susceptibility	Oxa23				P value
		Absent		Present		
		F	%	F	%	
MRP	Sensitive	57	100	0	0	
	Resistant	5	42	7	58	0.000
	Total	62	90	7	10	
CZX	Sensitive	39	100	0	0	
	Resistant	17	71	7	29	0.000
	Intermediate	6	100	0	0	
	Total	62	90	7	10	
CRO	Sensitive	10	100	0	0	
	Resistant	46	87	7	13	0.262
	Intermediate	6	100	0	0	
	Total	62	90	7	10	
CPM	Resistant	62	90	7	10	NA
	Total	62	90	7	10	

MRP: Meropenem; CZX: Ceftizoxime; CPM: Cefepime; CRO: Ceftriaxone.

Table 7. The correlation between the presence of oxa51 and antibiotics resistance.

Antibiotics	Susceptibility	oxa51				P. value
		Absent		Present		
		F	%	F	%	
MRP	Sensitive	57	100	0	0	
	Resistant	3	25	9	75	0.000
	Total	60	87	9	13	
CZX	Sensitive	39	100	0	0	
	Resistant	15	63	9	37	0.002
	Intermediate	6	100	0	0	
	Total	60	87	9	13	
CRO	Sensitive	10	100	0	0	0.388
	Resistant	44	83	9	17	

Continued

	Intermediate	6	100	0	0	
	Total	60	87	9	13	
CPM	Resistant	60	87	9	13	NA
	Total	60	87	9	13	

MRP: Meropenem; CZX: Ceftizoxime; CPM:Cefepime; CRO: Ceftriaxone.

Table 8. Presence of blaOXA-51 and blaOXA-23 in bacterial isolates.

Isolated Microorganism	Oxa51				Oxa23				Total
	Absent		Present		Absent		Present		
	F	%	F	%	F	%	F	%	
E. coli	34	92	3	8	34	92	3	8	37
S. aureus	18	86	3	14	20	95	1	5	21
P. mirabilis	9	90	1	10	10	100	0	0	10
K. pneumonia	5	83	1	17	5	83	1	17	6
P. aeruginosa	3	75	1	25	3	75	1	25	4
Total	69	88	9	12	71	91	7	9	78

5. Discussion

Urosepsis is a sepsis condition resulting from a dysregulated response to infections of urinary tract and/or male genital tract, its either community-acquired or healthcare associated infections remain in hospital, and needs medical intervention from starting antimicrobial therapy to the causes of the obstructive uropathy [4] this intervention was important risk factors of acquiring carbapenem resistant bacteria [15] [30]. This study highlights the incidence of carbapenem resistant genes, among *E. coli*, *K. pneumonia*, *P. mirabilis*, *P. Aeruginosa* and *S. aureus* isolated from urosepsis patients.

In this study males were more affected than females with average age 45 years old, because this study conducted in urology units at GHRDS and most admitted of patients suffering prostate cancer, benign prostatic hyperplasia (BPH) and urethral stricture, this result was agreed with study carried out by Ibrahim *et al.*, Goveas and Muhammad *et al.* study [31] [32] [33].

The isolates showed phenotypically high resistant rate to different groups of antibiotics this result similar to Ibrahim *et al.*, Ehssan studied in Sudan and Mohamed *et al.* studied in Cameroon, observed by using disc diffusion methods [31] [34] [35]. Globally, the outbreak of resistance to most prescribed antibiotics especially in complicated UTIs (cUTIs), urosepsis and pyelonephritis [36] [37], its led to exchange of mutant genes by intrinsic by transferable carbapenemase-encoding genes [12].

Most of these organisms observed contain ESBL genes [38], because carbapenem is the best option drug used to treat it [10]. Unfortunately, this study didn't

test forESBL genes about isolated bacteria, but Ibrahim *et al.* reported 45.1% from isolated bacteria containing ESBL genes in Khartoum teaching hospital, Sudan [26].

In this study the rate of CR phenotypically was 45.3%, this result is inagreement with CLSI and European committee on antimicrobial susceptibility testing standards were 17.4% and 10.9%, respectively [24]. The highest percentage of CR in this study as a result of being limited in β-lactam antibiotics used in urology department. According to MDR phenotypes, this study observes same frequency of isolated resistance among Latin America (41.1%) [24]. The detection of oxa23 and oxa51 in this was confirmed to the phenotypic resistance to carbapenem groups from isolated bacteria. Moreover, the detected genes observed in resistant strains to meropenem and was highly significant p value ($p = 0.000$).

The study finding the incidence of chromosomal and genes plasmid-mediated genes (blaOXA-51, blaOXA-23) were reported 13% and 10.1 respectively, it agrees with Mohamed *et al.* studied in the predominate gene but differs in the incidence, while the rate of blaOXA-51 gene and blaOXA-23 27.8% (10/36) and 2.7% (1/36), respectively [27].

Recently, some strains such as *P. Aeruginosa*, *K. pneumonia*, and *E. coli* appear the chromosomal genes blaOXA-51 and acquired blaOXA-23 gene among CRE these genes that appear only in *Acinetobacter* species [17] [27]. In this study CR gene (blaOXA-51, blaOXA-23) were found in *P. aeruginosa* 25% (1/4), *K. pneumonia* 17% (1/6), *S. aureus* 14% (3/18), *E. coli* 8% (3/37), *P. mirabilis* 10% (1/10) detected only in blaOXA-51 and the frequency of *S. aureus* in blaOXA-23 was 5% (1/18), although *E. coli* the predominant isolated, it has the lowest incidence in appearance of chromosomal genes blaOXA-51 among CRE [18] [23]. On the other hand, observed there is no study about these genes among *S. aureus*. Sample size played major role in the variation of the incidence and percentage between this study and Mohamed *et al.* study [27].

6. Conclusion

This study has shown a high rate of CR (blaOXA-23 and blaOXA-51) genes among the isolated bacteria collected from GHRDS, the *E. coli* was the predominant bacteria with the least frequency of blaOXA-23 and blaOXA-51 presence genes than other isolates. This result needs to focus on the higher frequency bacteria to CR gene especially *P. aeruginosa* and how to treat them without ignoring *E. coli*.

Study Limitation

This study is limited to imipenem and ertapenem as groups of carbapenem and conducted only in meropenem with third and fourth cephalosporin, and limited to ESBL genes in the detections.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Wang, S., Yuan, P., Peng, E., Xia, D., Xu, H., Wang, S., *et al.* (2020) Risk Factors for Urosepsis after Minimally Invasive Percutaneous Nephrolithotomy in Patients with Preoperative Urinary Tract Infection. *BioMed Research International*, **2020**, Article ID: 1354672. https://doi.org/10.1155/2020/1354672

[2] Ryan, J., O'Neill, E. and McLornan, L. (2021) Urosepsis and the Urologist! *Current Urology*, **15**, 39-44. https://doi.org/10.1097/CU9.0000000000000006

[3] Porat, A., Bhutta, B.S. and Kesler, S. (2022) Urosepsis. In: *StatPearls* [*Internet*], StatPearls Publishing, Treasure Island. http://www.ncbi.nlm.nih.gov/books/NBK482344/

[4] Bonkat, G., Cai, T., Veeratterapillay, R., Bruyère, F., Bartoletti, R., Pilatz, A., *et al.* (2019) Management of Urosepsis in 2018. *European Urology Focus*, **5**, 5-9. https://doi.org/10.1016/j.euf.2018.11.003

[5] Scotland, K. and Lange, D. (2018) Prevention and Management of Urosepsis Triggered by Ureteroscopy. *Research and Reports in Urology*, **10**, 43-49. https://doi.org/10.2147/RRU.S128071

[6] World Health Organization (2017, February 27) WHO Publishes List of Bacteria for Which New Antibiotics Are Urgently Needed. https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed

[7] Rodriguez, M. (2020, August 19) Whole Genome Analysis of Two Multiresistant *Escherichia coli* Strains. Effect of Grape Polyphenols on Bacterial Growth. *Enoforum*. https://www.enoforum.eu/en/poster2020/viticast-production-optimization-and-sustainable-development-of-the-vine-cultivation-through-fungal-diseases-prediction-feuga-viticast/

[8] Jesumirhewe, C., Springer, B., Allerberger, F. and Ruppitsch, W. (2020) Whole Genome Sequencing of Extended-Spectrum β-Lactamase Genes in *Enterobacteriaceae* Isolates from Nigeria. *PLoS ONE*, **15**, Article ID: e0231146. https://doi.org/10.1371/journal.pone.0231146

[9] Ur Rahman, S., Ali, T., Ali, I., Khan, N.A., Han, B. and Gao, J. (2018) The Growing Genetic and Functional Diversity of Extended Spectrum Beta-Lactamases. *BioMed Research International*, **2018**, Article ID: 9519718. https://doi.org/10.1155/2018/9519718

[10] Zango, U.U., Ibrahim, M., Shawai, S.A.A. and Shamsuddin, I.M. (2019) A Review on β-Lactam Antibiotic Drug Resistance. *Drug Design, Development and Therapy*, **3**, 52-58.

[11] Tshitshi, L., Manganyi, M.C., Montso, P.K., Mbewe, M. and Ateba, C.N. (2020) Extended Spectrum Beta-Lactamase-Resistant Determinants among Carbapenem-Resistant Enterobacteriaceae from Beef Cattle in the North West Province, South Africa: A Critical Assessment of Their Possible Public Health Implications. *Antibiotics*, **9**, Article No. 820. https://doi.org/10.3390/antibiotics9110820

[12] Codjoe, F.S. and Donkor, E.S. (2017) Carbapenem Resistance: A Review. *Medical Sciences*, **6**, Article No. 1. https://doi.org/10.3390/medsci6010001

[13] Dahab, R.A., Ibrahim, A.M. and Altayb, H.N. (2017) Phenotypic and Genotypic Detection of Carbapenemase Enzymes Producing Gram-Negative Bacilli Isolated from Patients in Khartoum State. *F1000Research*, **6**, Article No. 1656. https://doi.org/10.12688/f1000research.12432.1

[14] Smith, H.Z and Kendall, B. (2022) Carbapenem Resistant Enterobacteriaceae. In:

StatPearls, StatPearls Publishing, Treasure Island.
http://www.ncbi.nlm.nih.gov/books/NBK551704/

[15] Hansen, G.T. (2021) Continuous Evolution: Perspective on the Epidemiology of Carbapenemase Resistance among Enterobacterales and Other Gram-Negative Bacteria. *Infectious Diseases and Therapy*, **10**, 75-92.
https://doi.org/10.1007/s40121-020-00395-2

[16] Poirel, L., Naas, T. and Nordmann, P. (2010) Diversity, Epidemiology, and Genetics of Class D β-Lactamases. *Antimicrobial Agents and Chemotherap*, **54**, 24-38.
https://doi.org/10.1128/AAC.01512-08

[17] Wong, M.H., Chan, B.K., Chan, E.W. and Chen, S. (2019) Over-Expression of ISAba1-Linked Intrinsic and Exogenously Acquired OXA Type Carbapenem-Hydrolyzing-Class D-β-Lactamase-Encoding Genes Is Key Mechanism Underlying Carbapenem Resistance in *Acinetobacter baumannii*. *Frontiers in Microbiology*, **10**, Article No. 2809.
https://doi.org/10.3389/fmicb.2019.02809
https://www.frontiersin.org/article/10.3389/fmicb.2019.02809

[18] Mana, H.A., Sundararaju, S., Tsui, C.K.M., Perez-Lopez, A., Yassine, H., Thani, A.A., *et al.* (2021) Whole-Genome Sequencing for Molecular Characterization of Carbapenem-Resistant Enterobacteriaceae Causing Lower Urinary Tract Infection among Pediatric Patients. *Antibiotics*, **10**, Article No. 972.
https://doi.org/10.3390/antibiotics10080972
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8388976/

[19] Nordmann, P. and Poirel, L. (2019) Epidemiology and Diagnostics of Carbapenem Resistance in Gram-Negative Bacteria. *Clinical Infectious Diseases*, **69**, S521-S528.
https://doi.org/10.1093/cid/ciz824

[20] Evans, B.A. and Amyes, S.G.B. (2014) OXA β-Lactamases. *Clinical Microbiology Reviews*, **27**, 241-263. https://doi.org/10.1128/CMR.00117-13

[21] Queenan, A.M. and Bush, K. (2007) Carbapenemases: The Versatile β-Lactamases. OXA β-Lactamases. *Clinical Microbiology Reviews*, **27**, 241-263., **20**, 440-458.
https://doi.org/10.1128/CMR.00001-07

[22] Segagni Lusignani, L., Presterl, E., Zatorska, B., Van den Nest, M. and Diab-Elschahawi, M. (2020) Infection Control and Risk Factors for Acquisition of Carbapenemase-Producing Enterobacteriaceae. A 5 Year (2011-2016) Case-Control Study. *Antimicrobial Resistance & Infection Control*, **9**, Article No. 18.
https://doi.org/10.1186/s13756-019-0668-2

[23] Zahedi bialvaei, A., Samadi kafil, H., Ebrahimzadeh Leylabadlo, H., Asgharzadeh, M. and Aghazadeh, M. (2015) Dissemination of Carbapenemases Producing Gram Negative Bacteria in the Middle East. *Iranian Journal of Microbiology*, **7**, 226-246.

[24] Tsilipounidaki, K., Athanasakopoulou, Z., Müller, E., Burgold-Voigt, S., Florou, Z., Braun, S.D., *et al.* (2022) Plethora of Resistance Genes in Carbapenem-Resistant Gram-Negative Bacteria in Greece: No End to a Continuous Genetic Evolution. *Microorganisms*, **10**, Article No. 159.
https://doi.org/10.3390/microorganisms10010159

[25] Brink, A.J. (2019) Epidemiology of Carbapenem-Resistant Gram-Negative Infections Globally. *Current Opinion in Infectious Diseases*, **32**, 609-616.
https://doi.org/10.1097/QCO.0000000000000608

[26] Ibrahim, M.E., Bilal, N.E., Magzoub, M.A. and Hamid, M.E. (2013) Prevalence of Extended-Spectrum β-Lactamases-Producing *Escherichia coli* from Hospitals in Khartoum State, Sudan. *Oman Medical Journal*, **28**, 116-120.
https://doi.org/10.5001/omj.2013.30

[27] Mohamed Elmutasim, A.M., Abdalla, O.A., El-Shiekh, B.K., Bashir, S. and Musa, A.A. (2019) Phenotypic and Genotypic Characterization of Carbapenemsresistant clinical Gram-Negative Bacteria from Sudan. *Edorium Journal of Microbiology*, **5**, Article ID: 100011M08MM2019. http://edoriumjournalofmicrobiology.com/archive/article-full-text/100011M08MM2019

[28] Chen, Y.-L., Lee, C.-C., Lin, Y.-L., Yin, K.-M., Ho, C.-L. and Liu, T. (2015) Obtaining Long 16S rDNA Sequences Using Multiple Primers and Its Application on Dioxin-Containing Samples. *BMC Bioinformatics*, **16**, Article No. S13. https://doi.org/10.1186/1471-2105-16-S18-S13

[29] Joshi, P.R., Acharya, M., Kakshapati, T., Leungtongkam, U., Thummeepak, R. and Sitthisak, S. (2017) Co-Existence of blaOXA-23 and blaNDM-1 Genes of *Acinetobacter baumannii* Isolated from Nepal: Antimicrobial Resistance and Clinical Significance. *Antimicrobial Resistance & Infection Control*, **6**, Article No. 21. https://doi.org/10.1186/s13756-017-0180-5

[30] Bhojani, N., Miller, L.E., Bhattacharyya, S., Cutone, B. and Chew, B.H. (2021) Risk Factors for Urosepsis After Ureteroscopy for Stone Disease: A Systematic Review with Meta-Analysis. *Journal of Endourology*, **35**, 991-1000. https://doi.org/10.1089/end.2020.1133

[31] Ibrahim, S.O., Elimam, M., Taha, S.M., Yousif, S.M., Omer, H.M.H., Yousif, M., *et al.* (2022) Urosepsis among Sudanese Patients: A Paradigm from Limited Resources Country. *Advances in Microbiology*, **12**, 109-120. https://doi.org/10.4236/aim.2022.123010

[32] Goveas, B. (2017) Urosepsis: A Simple Infection Turns Toxic. *The Nurse Practitioner*, **42**, 53-54. https://doi.org/10.1097/01.NPR.0000520425.91534.b8

[33] Muhammad, A.S., Onwuasoanya, U.E., Mohammad, Y., Agwu, N.P., Abdulwahab-Ahmed, A. and Mungadi, I.A. (2021) Presentation, Risk Factors, Microbiological Pattern and Management of Urosepsis in a Tertiary Hospital in Nigeria. *Basic Research Journal of Medicine and Clinical Sciences*, **1**, 1-7.

[34] Mouiche, M.M.M., Moffo, F., Akoachere, J.-F.T.K., Okah-Nnane, N.H., Mapiefou, N.P., Ndze, V.N., *et al.* (2019) Antimicrobial Resistance from a One Health Perspective in Cameroon: A Systematic Review and Meta-Analysis. *BMC Public Health*, **19**, Article No. 1135. https://doi.org/10.1186/s12889-019-7450-5

[35] Moglad, E.H. (2020) Antibiotics Profile, Prevalence of Extended-Spectrum Beta-Lactamase (ESBL), and Multidrug-Resistant Enterobacteriaceae from Different Clinical Samples in Khartoum State, Sudan. *International Journal of Microbiology*, **2020**, Article ID: 8898430. https://doi.org/10.1155/2020/8898430

[36] Wagenlehner, F.M.E., Bjerklund Johansen, T.E., Cai, T., Koves, B., Kranz, J., Pilatz, A., *et al.* (2020) Epidemiology, Definition and Treatment of Complicated Urinary Tract Infections. *Nature Reviews Urology* **17**, 586-600. https://doi.org/10.1038/s41585-020-0362-4

[37] Ladjevic, N., Vuksanovic, A., Durutovic, O., Jovicic, J., Ladjevic, N., Likic-Ladjevic, I., *et al.* (2021) Urosepsis in Adults. *Archives of Biological Sciences*, **73**, 205-214. https://doi.org/10.2298/ABS210304015L

[38] Abdelaziz, N.A. (2022) Phenotype-Genotype Correlations among Carbapenem-Resistant Enterobacterales Recovered from Four Egyptian Hospitals with the Report of SPM Carbapenemase. *Antimicrobial Resistance & Infection Control*, **11**, Article No. 13. https://doi.org/10.1186/s13756-022-01061-7