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Frequency of Multi-Drug Resistant *Enterobacter* Species Isolated from Patients with Different Clinical Manifestations in Khartoum State, Sudan

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ABSTRACT

Enterobacter species are members of ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter species*) which are described as the leading cause of resistant nosocomial infections. **Objectives:** The present study aimed to estimate the frequency of Multi-Drug resistant *Enterobacter* species isolated from Patients with different Clinical manifestations in Khartoum state, Sudan. **Methods:** A cross-sectional laboratory-based study was conducted from February 2021 to October 2021. To isolate and identify *Enterobacter* species from different clinical specimens by conventional cultural methods, and to determine the antimicrobial profile of *Enterobacter* species by Kirby–Bauer disc diffusion technique. **Result:** A total of three hundred and eighty-four (n=384) different clinical specimens (urine 232(60%), wound swab 61 (16%), sputum 19(5%), and blood 72 (19%) specimens were collected from Yastabshiroon Hospital, Khartoum, 129(33.6%), Ribat Teaching Hospital, Khartoum, 255(66.4%), 173 (45.0%) were from males, while 212 (55.0%) from females, among 154(40%) bacterial isolate 22(14.3%) were identified as *Enterobacter* spp. All *Enterobacter* spp isolates (100%) were multidrug resistant, The Prevalence rates of resistance of the isolates among antibiotic classes were as follows; (Penicillin 100%, Cephalosporin 96.4%, Tetracycline group 100%, Aminoglycosides 47.7%, Sulfonamides and trimethoprin 86.4%, Nitrofurantoin 100%, Quinolone 72.75%, Glycopeptides 100%, Chloramphenicol 100%, Tetracycline 81.8% and Carbapenem 52.3%). **Conclusion:** This study is the first study reporting the frequency of Multi-drug resistance *Enterobacter* species in Sudan. The results of this study indicated the high prevalence of *Enterobacter* species resistant to the majority of assessed antibiotics, and high prevalence rates of carbapenemase and ESBL-producing *Enterobacter* species In Khartoum- Sudan.

INTRODUCTION

The genus *Enterobacter* includes facultative anaerobic Gram-negative bacilli that are 2 mm long, motile by peritrichous flagella and belong to the family *Enterobacteriaceae*. Several strains of these bacteria are pathogenic and cause opportunistic infections in immunocompromised (Adeolu *et al.*, 2016). It was first described in 1960, but changes in taxonomy have occurred in the last 50 years (Anne P *et al.*, 2019). For example, *E. sakazakii* has been reassigned to a new genus *Cronobacter* (Anne P *et al.*, 2019).

Enterobacter spp are natural commensals of the animal and human gut microbiota. Among these bacteria, only certain subspecies/species have been associated with hospital-acquired infections and outbreaks (Akbari M *et al.*, 2015).

Enterobacter infections can include bacteremia, lower respiratory tract infections, skin and soft-tissue infections, urinary tract infections, endocarditis, intra-abdominal infections, septic arthritis, osteomyelitis, central nervous system infections, and ophthalmic infections (Mezzatesta *et al.*, 2012). Urinary and respiratory tracts are the most common sites of infection. *Enterobacter* infections do not produce a unique enough clinical presentation to differentiate them clinically from other acute bacterial infections (Mezzatesta *et al.*, 2012).

Enterobacter species are members of ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* species) which are described as the leading cause of resistant nosocomial infections (Akbari M *et al.*, 2015), (Wu W, Feng Y, Zong Z. 2018). *Enterobacter aerogenes*, *E. cloacae* and *E. hormaechei* represent the most frequently isolated species described in clinical infections, especially in immunocompromised patients and those hospitalized in an ICU (Intensive Care Unit), due to the adaptation of these species to

antimicrobial agents and their behavior as opportunistic pathogens. Several hospital outbreaks have been reported in Europe since the mid-1990s, and the wide use of extensive broad-spectrum antibiotics has stimulated the spread of resistant clones (Anne P *et al.*, 2019). These pathogens are frequently associated with a multidrug-resistance phenotype, mainly due to their adaptation to the hospital environment and the pathogens' ability to easily acquire numerous genetic mobile elements containing resistance and virulence genes, which make their treatment difficult (Davin-Regli A, Pagès JM. 2015), (Davin -Regli A, Masi M, *et al.*, 2016). Antibiotic resistance, regulation of resistant genes and the clinical implications of these situations have been extensively studied (Anne P *et al.*, 2019).

The accurate identification of species and subspecies remains a challenge. The development of genome sequencing has rapidly modified the phylogeny of the genus, particularly that of the *E. cloacae* complex (Izdebski R *et al.*, 2015).

Pathogenicity/virulence of this bacterium remains rather unclear due to the limited number of works performed to date in this field. In contrast, its resistance against antibacterial agents has been extensively studied. In the face of antibiotic treatment, it is able to manage different mechanisms of resistance *via* various local and global regulator genes and the modulation of the expression of different proteins, including enzymes (β -lactamases, etc.) or membrane transporters, such as porins and efflux pumps (Anne P *et al.*, 2019).

β -lactam antibiotics, third-generation cephalosporins and carbapenems, are used to treat infections caused by several species of *Enterobacter* (Peymani *et al.*, 2014). β -lactamase enzymes, including extended-spectrum β -lactamases (ESBLs) and AmpC, are involved in the mechanism underlying resistance to β -lactam antibiotics in *Enterobacter* spp (Mohd Khari *et al.*, 2016).

MATERIALS AND METHODS

Study Design, Duration, and Population:

A cross-sectional laboratory-based study was conducted from February 2021 to October 2021. This study was conducted at Yastabshiroon Hospital and Ribat Teaching Hospital in Khartoum State, Sudan. The specimen was collected from Sudanese patients who were expected to have bacterial infections and had been examined by a physician. A total of three hundred and eighty-four (n=384) different clinical specimens (urine, wound swab, sputum, and blood) were collected in sterile containers.

Sampling Technique:

This study is based on the no-probability convenience sampling technique.

Ethical Clearance:

The study proposal was approved by the ethical board of Sudan University of Science and Technology and Research Committee, Ministry of Health. Informed consent was taken from each patient before enrollment into the study.

Isolation and Identification Schemes:

MacConkey agar media and Chromogenic agar media (Liofilchem Co. Italy) were used for the isolation of *Enterobacter* isolates. The medium was prepared and the plate was inoculated from urine, wound swabs, sputum specimens, and blood culture media, then the plates were incubated overnight at 37 °C. The isolate was identified according to lactose fermentation on MacConkey agar and the color produced on Chromogenic media. The *Enterobacter* species was lactose fermented and produced pink color in MacConkey agar media and produced green color in Chromogenic agar media (Liofilchem Co. Italy).

The Gram's stain and Biochemical tests for Gram-negative bacteria (Catalase test, Oxidase test, kiligler Iron ager test, Motility test, Citrate utilization test, Urea hydrolysis test, and Indol test) were used to confirm the identity of isolates. Isolates were identified based on colonial morphology, motility, Gram's staining, oxidase and biochemical reactions.

Antimicrobial Susceptibility test Enterobacter Species:

The susceptibility of isolates was determined by the agar diffusion method, using a modification of the Kirby-Bauer disc diffusion technique according to CLSI guidelines (CLSI, 2018). The following antibiotic discs (drug concentrations in µg) were used: Amikacin (30), Amoxicillin (30), Amoxyclav (30), Ciprofloxacin (5), Ceftriaxone (30), Cephotoxime (30), Ceftazidime (30), Cefotaxime (30), Cefepime (30), Chloramphenicol (30), Co-trimoxazole (30), Nitrofurantoin (30), Vancomycin (30), Tetracycline (30), Gentamicin (30), Imipenem (30), and Colistin. *Enterobacter*-like isolates from an overnight culture were used for the sensitivity test. A colony of the isolate was picked with a straight sterile wire, inoculated into sterile 5 ml peptone water, and shaken to dissolve it. The turbidity of the suspension was matched against 0.5 McFarland standards. Poured on a sterile Mueller-Hinton agar plate. The inoculated agar plate was swayed gently to ensure that the whole agar surface was covered. The plate was drained to remove excess fluid. The antibiotic discs were placed on the agar surface, leaving a space of 25mm between them, and pressed slightly to ensure sufficient contact with the agar surface. The plates were then incubated at 37 °C for 24 h. The plates were incubated inverted in the incubator at 37°C overnight. The plates were examined for inhibition zone size. Positive control (*Enterobacter* ATCC25922) and negative control (*E. coli* ATCC25922) were used to confirm the results.

Extended-spectrum β-lactamases (ESBLs) Confirmatory Test:

The presence of ESBLs was confirmed by Phenotypic Confirmatory Test method (PCT). The CLSI-ESBL phenotypic confirmatory test with Ceftazidime 30 µg, Cefotaxime 30 µg and Cefepime 30 µg was performed for all positive isolates by disc diffusion method on Mueller-Hinton agar plates with and without 10 µg of Clavulanic acid. A tube containing about 2 ml of sterile normal saline is inoculated with a pure culture

growth until matching has occurred with 0.5 McFarland turbidity standard of approximately $1-2 \times 10^8$ CFU/ml. For each isolate one or more of the following cephalosporins disc ceftazidime (CAZ) 30 µg, cefotaxime (CTX) 30 µg and cefepime (FEP) was added to Mueller-Hinton agar plate seeded with bacterial suspension. On the same plate one or more of the antibiotics discs containing ceftazidime 30 µg+10 µg of clavulanic acid (CAL 30/10 µg), cefotaxime 30 µg+10 µg of clavulanic acid (CTL 30/10 µg) and cefepime 30 µg+10 µg of clavulanic acid (supplied by Liofilchem Co. Italy), were applied aseptically on the plate and pressed gently to agar surface using a sterile forceps. Within 15 min the plates were incubated at 37 °C for 16-18 hrs. A difference of ≥ 5 mm between the zone diameters of either of the cephalosporin disk and their respective cephalosporin/clavulanate disk is taken to be phenotypic confirmation of ESBLs production.

Modified Hodge Test:

The presence of carbapenemase enzyme was confirmed by Modified Hodge Test (MHT). A 1:10 dilution of inoculums of the indicator organism *E. coli* ATCC 25922, adjusted to a 0.5 McFarland turbidity standard, was used to inoculate the surfaces of plates containing Mueller-Hinton agar by swabbing. After the plates were allowed to stand at room temperature for 10 min, a disc containing meropenem (10 µg) were placed on the agar plates. Subsequently, three to five colonies of the test organisms (from an agar plate grown overnight) were inoculated onto the plate in a straight line out from the edge of

the disk, using a loop. Quality control of the following organisms MHT Positive *Klebsiella pneumoniae* ATCC1705 and MHT Negative *Klebsiella pneumoniae* ATCC1706 were run with each batch of the test.

Plates were examined after overnight incubation at 37°C. After 24 hrs, MHT Positive test showed a clover leaf-like indentation of the *Escherichia coli* 25922 growing along the test organism growth streak within the disk diffusion zone. MHT Negative test showed no growth of *Escherichia coli* 25922 along the test organism growth streak within the disk diffusion.

Data Analysis:

Data were analyzed using the statistical package for social science software (SPSS v. 20). A *p*-value of <0.05 was considered significant for all statistical tests in the present study.

RESULTS

A total of three hundred and eighty-four (n=384) different clinical specimens (urine 232(60%), wound swab 61 (16%), sputum 19(5%), and blood 72 (19%) as shown in figure 1. Specimens were collected from different Hospitals, these include Yastabshiroon Hospital, Khartoum 129(33.6%), Ribat Teaching Hospital, Khartoum 255(66.4%), 173 (45.0%) were Males, while 212 (55.0%) were females, 218(56.8%) were outpatients, 142(37%) were inpatients, 20(5.2%) ICU patients, and 4(1.0%) NICU patients. The age of patients included in this study ranged from 2 days to 90 years classified into five categories.

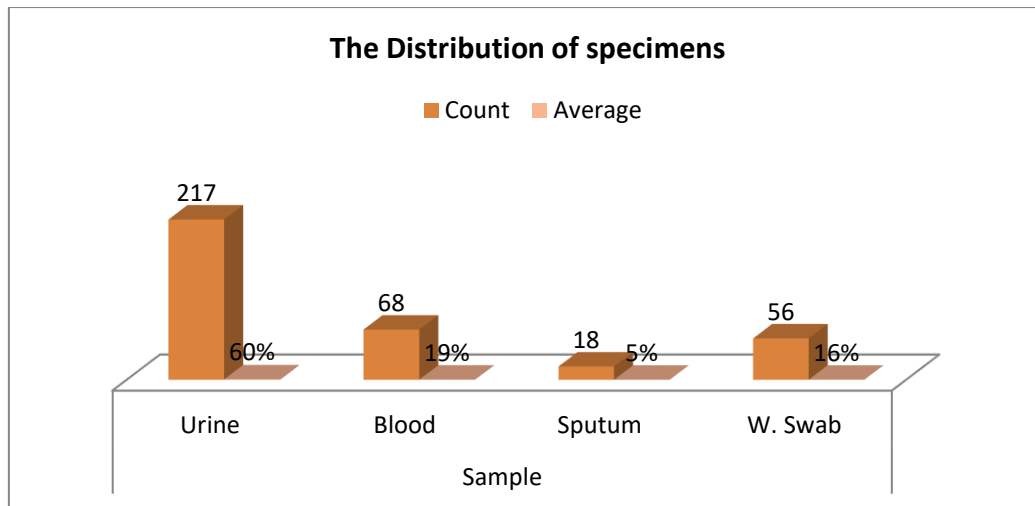


Fig 1: The distribution of specimens.

Bacterial Isolates:

Identification of the isolates was carried out by using colony morphology on MacConkey agar media following incubation for 24 h at 37 °C, colonies were circular (1–2

mm in diameter), white, Opaque and elevated with regular margin lactose fermented, and on Chromogenic agar media Produced green color. As shown in Figure 2.

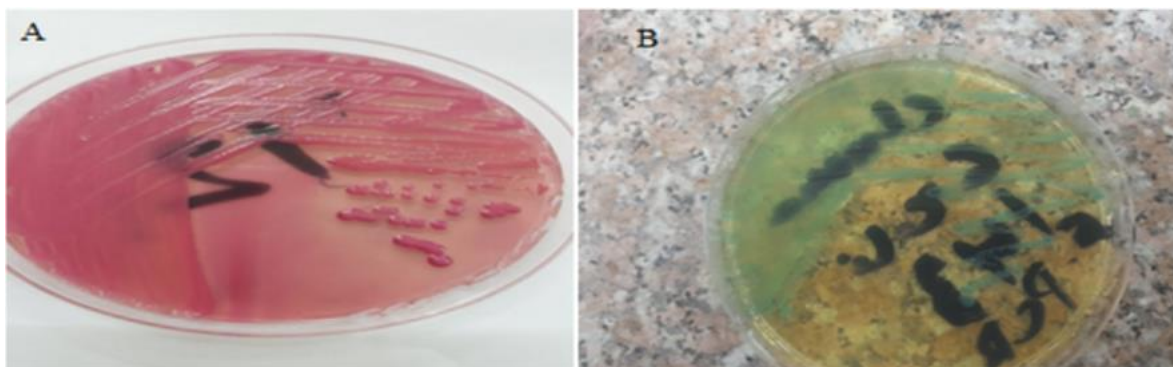


Fig. 2: Colonial morphology of isolated bacteria on MacConkey agar and Chromogenic agar media.

A. Lactose fermented colonies produced by *Enterobacter* spp on MacConkey agar media.

B. *Enterobacter* spp produced green color colonies on Chromogenic agar media.

Gram's Stain and Biochemical Tests:

Microscopic examination showed that these bacteria were Gram-negative rod-shaped (0.562–3 mm) and occurred singly, as shown in Figure 3-A.

Basic biochemical characterization of the 22 *Enterobacter*-like isolates was achieved using the Catalase test, Oxidase test, kiligler Iron ager test, Motility test, Citrate Utilization test, Urea hydrolysis test, and Indol test, as shown in Figure 3-B. Catalase activity was determined by observing bubble formation

after dropping H₂O₂ on 24 hrs old biomass on nutrient agar. Oxidase activity was tested by using an oxidase reagent (bioMe'rieux).in this study showed that all *Enterobacter*-like isolates were motile, catalase positive, and oxidase negative, lactose and glucose fermented with gas and noH₂s production on kiligler Iron ager. While 21(95.4%) isolates were Indol (negative), 16(72.7%) isolates were Citrate (positive), and 16 (72.7%) isolates were Urease (negative).

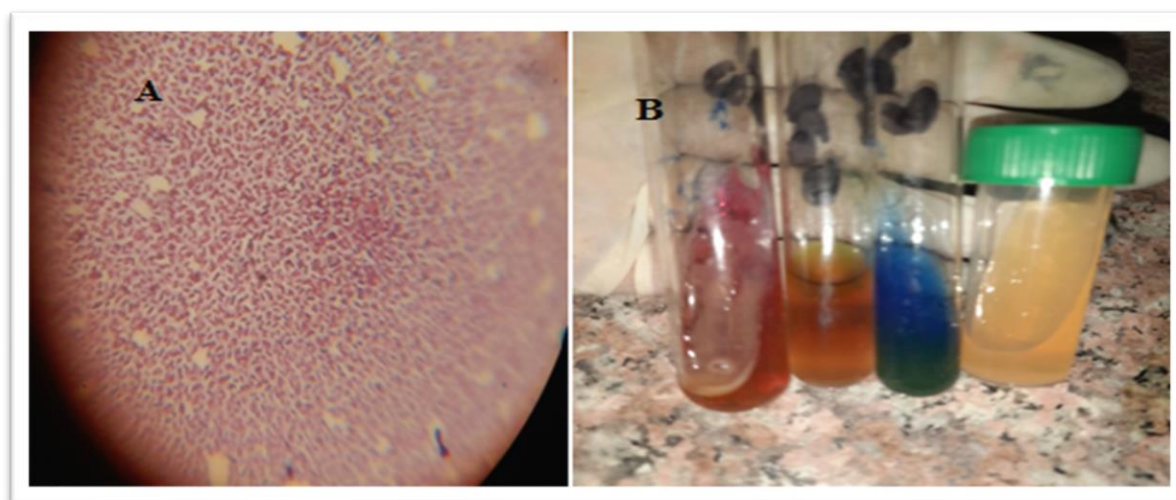


Fig. 3: A: showing Gram-negative rod-shaped (0.562–3 mm) and occurred singly. B: 24 hr old culture of isolated bacteria on kiligler Iron agar test show lactose and glucose fermented with gas and no production of H₂s, Citrate Utilization test (positive), Urea Hydrolysis Test (negative), and Indol test (negative).

Antimicrobial Susceptibility Test:

All the 22 *Enterobacter* spp isolates were resistant to Amoxicillin, Amoxyclav, Ceftriaxone, Cephataxime, Ceftazidime, Cefotaxime, Chloramphenicol, Nitrofurantoin, and Vancomycin (100%). While 21(95.5%) isolates were sensitive to Amikacin, 4 (18.2%) to Cefepime and Tetracycline, 3(13.6%) to Co-trimoxazole, 9(40%) to Ciprofloxacin, 2(9.1%) to Gentamicin, 8 (36.4%) to Imipenem, and 13(59.1%) to Colistin, as exhibited in Table 1.

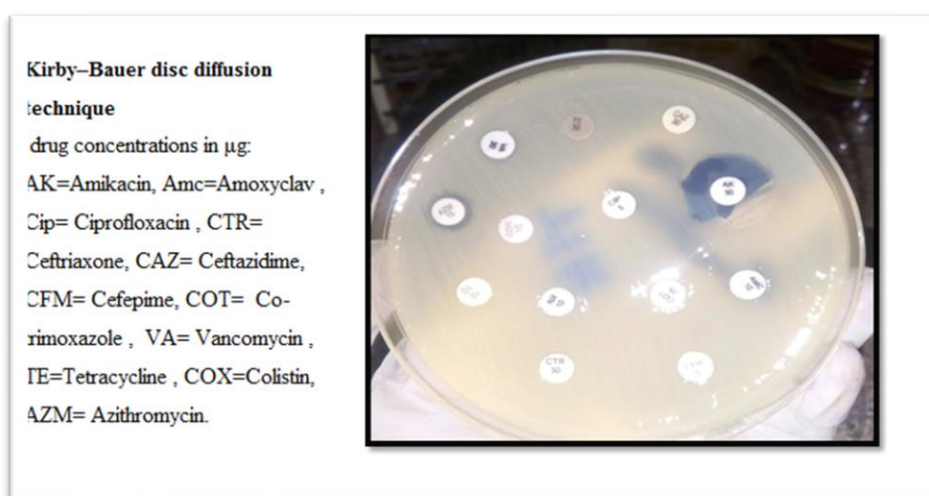
This study showed that all *Enterobacter* spp isolates (100%) were

multidrug-resistant, The Prevalence rates of resistant isolates among antibiotic class were (Penicillin 100%, Cephalosporin 96.4%, Tetracycline group 100%, Aminoglycosides 47.7%, Sulfonamides and trimethoprim 86.4%, Nitrofurantoin 100%, Quinolone 72.75%, Glycopeptides 100%, Chloramphenicol 100%, Tetracycline 81.8% and Carbapenem 52.3%).

In this study, we have shown a high prevalence of ESBLs and carbapenemase enzymes produced by *Enterobacter* spp in Khartoum.

Table 1: Antimicrobial susceptibility profiles the isolates of *Enterobacter*-spp

Frequency of MDR <i>Enterobacter</i> spp among Antibiotic classes	Antimicrobial agent	Sensitive	Resistant
		Frequency (%)	Frequency (%)
Penicillin (100%)	Amoxicillin	0 (0.0%)	22(100%)
	Amoclave	0(0.0%)	22(100%)
Cephalosporin (96.4%)	Cefotaxime	0(0.0%)	22(100%)
	Ceftazidime	0(0.0%)	22(100%)
	Ceftriaxone	0(0.0%)	22(100%)
	Cefepime	4(18.2%)	18(81.8%)
	Cephoxime	0(0.0%)	22(100.0%)
Sulfonamides and trimethoprin (86.4%)	Co-trimoxazole	3(13.6%)	19(86.4%)
Aminoglycosides (47.7%)	Amickacin	21(95.5%)	1(4.5%)
	Gentamicin	2(9.1%)	20(90.9%)
Nitrofurantoin (100%)	Nitrofurantoin	0(0.0%)	22(100.0%)
Quinolone (72.75%)	Ciprofloxacin	11(50.0%)	11(50.0%)
	Norofloxacin	1(4.5%)	21(95.5%)
Glycopeptides (100%)	Vancomycin	0(0.0%)	22(100.0%)
Chloramphenicol (100%)	Chloramphenicol	0(0.0%)	22(100.0%)
Tetracycline (81.8%)	Tetracycline	4(18.2%)	18(81.8%)
Carbapenem (52.3%)	Imipenem	8(36.4%)	14(63.6%)
	Colistin	13(59.1%)	9(40.9%)

**Fig. 4:** Antimicrobial Susceptibility test by Kirby–Bauer disc diffusion technique.

Demographic Data of Patients with *Enterobacter* Spp Infections:

Among the 384 different clinical specimens, 22 isolates identified as *Enterobacter* spp, 11 (50%) were males while 11 (50%) were females. There was no significant difference in the distribution of isolates among the gender ($p = 0.645$).

The ages of patients were classified into five categories: the highest frequency was shown in the age group (15-30 years old, 46-60 years old, and age group >60 years old 6 (27.3%) the lowest frequency was shown in the age group <15 years old 1(4.5%), There was no significant difference in the distribution of isolates among the age group ($p = 0.246$). as shown in Table2.

Table 2: shows the demographic data of a patient with *Enterobacter* infection, including age and gender

Demographic data									
Gender				Age groups					
Gender	Frequency	Percent	P value	Age groups	Frequency	Percent	P value	Mean	Std. Deviation
Male	11	50%	0.645	>15	1	4.5%	0.246	48.6	23.1
				16-30	6	27.3%			
Female	11	50%		31-45	3	13.6%			
				46-60	6	27.3%			
				>60	6	27.3%			
Total	22	100%				22			

Table 3 shows the distribution of *Enterobacter* spp among patient status, the frequency of patients suffering in different clinics (outpatient) was 7(31.8%), while 12(54.5%) were admitted to hospital(inpatient), and 3(13.6%) ICU patient. There was no significant difference in the distribution of isolates among the patient status group ($p = 0.259$).

Table 3: Distribution of Patient status.

Patient status	Frequency	Percent	P value
Outpatient	7	31.8%	0.259
Inpatient	12	54.5%	
ICU	3	13.6%	
Total	22	100.0%	

Frequency of Multi-Drug Resistant *Enterobacter* spp Infections Among Significant Growth:

A total of 384 (100%) clinical specimens were inoculated on MacConkey agar media and chromogenic agar media, 230 (60%) was no growth, while 154(40%) significant bacterial growth, the result showed a high frequency of *Enterobacter* spp 22(14%) {(9 (5.8%) urine, 11(7%) wound swab, 2(1.2%) sputum, 0(0) % blood)} among 154(40%) significant bacterial growth as shown in Table 4.

Table 4: Frequency of *Enterobacter* spp infections among significant growth.

Diagnosis	Specimen	Total (%) of Significant growth	<i>Enterobacter</i> spp
UTI	Urine	83 (54%)	9(5.8%)
Wound infection	Wound Swab	47(30.5%)	11(7.0%)
Pneumonia	Sputum	10(6.5%)	2(1.2%)
Septicemia	Blood	14(9.0%)	0 (0%)
Total (%)		154(100%)	22(14.0%)

Frequency of Multi-Drug Resistant *Enterobacter* spp Among Other Isolated Bacteria:

Among 154 bacterial isolate 22(14.3%) were confirmed as *Enterobacter* spp, while *E. coli* 38(24.7%), *Klebsiella* spp 10(12.3). *Staphylococcus* spp 16(10.4%), *Proteus* spp 10(6.5%), and *Pseudomonas* spp

21(13.6%), *E.fecales* 13(8.4%), *Candida* spp 8(5.1%), and *Moraxella* spp 2(1.3%), *Aceintobacter* 2(1.3), *Serratia*, *Citrobacter*, and *S.pyogen* 1(0.7%). As many as 28(18.2 %) other species, as shown in Figure 5. *Enterobacter* spp is the second most common cause of bacterial infections.

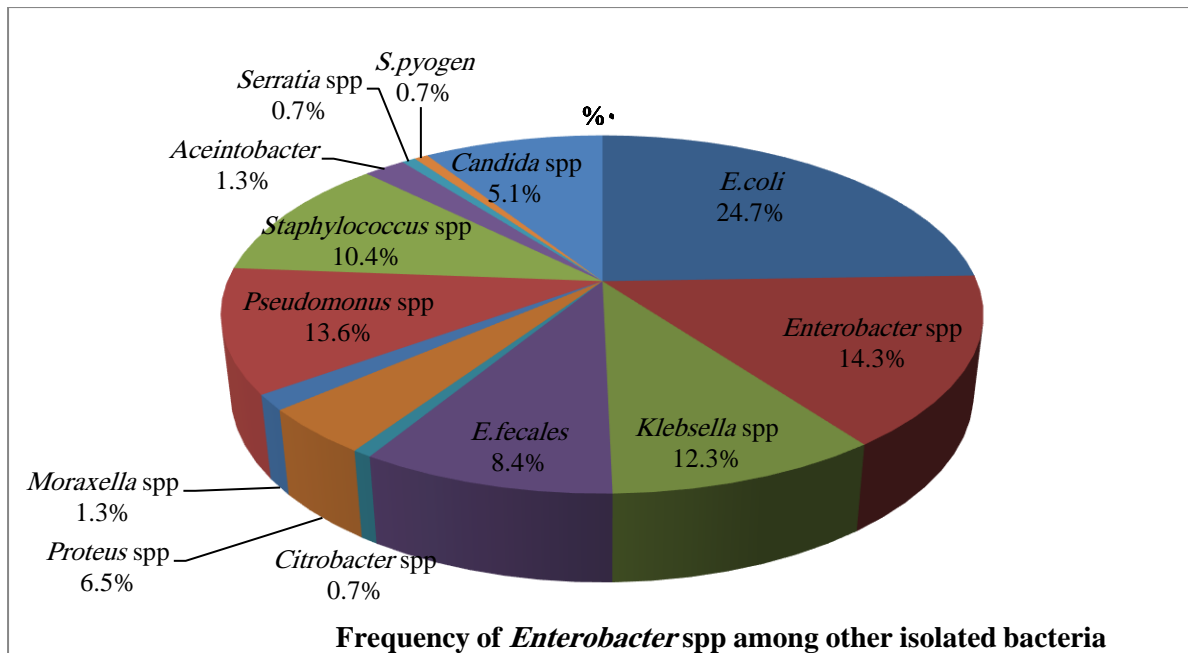


Fig. 5: Frequency of Multi-Drug resistant *Enterobacter* spp among other isolated bacteria.

Initial investigation showed that the etiological agents were Gram-stain-negative, rod-shaped isolates that possessed most of the morphological and phenotypic characteristics of the genus *Enterobacter*, but could not be unambiguously assigned to any currently known species.

DISCUSSION

Enterobacter species have been recognized as increasingly important pathogens in recent years. They have increased in incidence as causes of nosocomial infections in general (the third most common cause of nosocomial Gram-negative bacillary respiratory infections; as with other aerobic Gram-negative rods), (Mezzatesta *et al.*, 2012).

In this study a total of three hundred and eighty-four (n=384) different clinical specimens (urine 232(60%), wound swab 61 (16%), sputum 19(5%), and blood 72 (19%). Specimens were collected from different Hospitals in Khartoum-Sudan, these include Yastabshiroon Hospital 129(33.6%), Ribat Teaching Hospital 255(66.4%), 173 (45.0%) were Males, while 212 (55.0%) were females. Among 154(40%) bacterial isolate 22(14.3%) were confirmed as *Enterobacter* spp, show 11 (50%) were males, and 11(50%) were

females; statistically there was no significant association (P -value > 0.05) between *Enterobacter* infections and gender ($p = 0.645$). The ages of patients in this study were classified into five categories: the highest frequency was shown in the adult groups including age group (15-30 years old, 46-60 years old, and age group >60 years old 6 (27%) the lowest frequency was shown in the children group <15 years old 1(4.5%). There was no significant association between *Enterobacter* infections and age groups ($p = 0.246$). These findings agree with a previous study conducted in Nigeria by (Mordi and Hugbo.2011) who recorded there was no significant difference in the distribution of isolates among the age groups ($p > 0.05$).

This study showed that the distribution of *Enterobacter* species isolates was 12(54.5%) in admitted hospital patients (inpatients) including intensive care unit patients 3(13.6%), while 7(31.8%) of outpatients accounted for the least frequent samples, There was no significant difference in the distribution of isolates among the patient status group ($p = 0.259$), these findings disagree with a previous study conducted in Kermanshah, Iran by (Zeinab Mohseni *et al*; 2021) who recorded most cases of positive

Enterobacter were isolated from ICUs (ICU, NICU, and PICU; 35.6%) and the emergency ward (bone marrow and kidney; 1.3%) and outpatients (9.2%) accounted for the least frequent samples.

In this study, most cases of positive *Enterobacter* were isolated from wound swab (11(7%)), urine 9 (5.8%), sputum 2 (1.2%), 0(0%) blood, these findings disagree with a previous study conducted in Kermanshah, Iran by (Zeinab Mohseni *et al.*; 2021) who recorded most cases of positive *Enterobacter* were isolated from urine (51.6%) and sputum samples (20.5%), while the positive cases isolated from vaginal and tissue biopsy samples (0.2%) were least frequent.

This study shows all *Enterobacter* spp isolates were resistant to Amoxicillin, Amoxyclav, Ceftriaxone, Cephotaxime, Ceftazidime, Cefotaxime, Chloramphenicol, Nitrofurantoin, and Vancomycin (100%). While (95.5%) isolate was sensitive to Amikacin, (18.2%) isolates were sensitive to Cefepime and Tetracycline, (13.6%) isolates were sensitive to Co-trimoxazole, (40%) isolates were sensitive to Ciprofloxacin, (9.1%) isolates were sensitive to Gentamicin, while (36.4%) isolates were sensitive to Imipenem, and (59.1%) isolates were sensitive to Colistin, In this study show all *Enterobacter* spp were multidrug resistant isolate (100%), the Prevalence rates of resistant isolate among antibiotic class were (Penicillin 100%, Cephalosporin 96.4%, Tetracycline group 100%, Aminoglycosides 47.7%, Sulfonamides and trimethoprim 86.4%, Nitrofurantoin 100%, Quinolone 72.75%, Glycopeptides 100%, Chloramphenicol 100%, Tetracycline 81.8% and Carbapenem 52.3%). these findings agree with a previous study conducted in Sudan by (Saeed and Musallam, 2011) who recorded that out of 389 specimens examined, 6 (1.5%) *E.sakazakii* was recovered. The results moreover revealed that the antimicrobial resistance of *E.sakazakii* was as follows; ceftazidime, amoxicillin, amoxyclav (100% each), co-trimoxazole, ticarcycline (83.3% each), chloramphenicol, tetracycline, ceftriaxone, nitrofurantoin,

cephotaxime, tobramycin (66.7% each), ciprofloxacin, amikacin and nalidixic acid (16.7% each). None of the isolates were found to be resistant to gentamicin. The results indicated for the first time the presence of *E.sakazakii* in the examined clinical specimens in Sudan. The occurrence was high and the antimicrobial resistance of the isolated *E.sakazakii* was also high. Also, these findings disagree with a previous study conducted in Iran by (Farzad Khademi *et al.* 2022) who recorded pooled prevalence of *Enterobacter* species resistant to various antibiotics as follows: Imipenem 16.6%, Meropenem 16.2%, Aztreonam 40.9%, Ciprofloxacin 35.3%, Norfloxacin 31%, Levofloxacin 48%, Gentamicin 42.1%, Amikacin 30.3%, Tobramycin 37.2%, Tetracycline 50.1%, Chloramphenicol 25.7%, Timethoprim/Sulfamethoxazole 52%, Nalidixic acid 49.1%, Nitrofurantoin 43%, Ceftriaxone 49.3%, Cefixime 52.4%, Cefotaxime 52.7%, Ceftazidime 47.9%, Cefepime 43.6%, and Ceftizoxime 45.5%). Prevalence rates of MDR and ESBL-producing *Enterobacter* species in Iran were 63.1% and 32.8%, respectively.

Conclusion

Enterobacter spp has emerged as a clinically significant cause of a wide variety of bacterial infections. There is still a lack of comprehensive molecular and clinical epidemiological analysis.

In this study, *Enterobacter* spp is the second most common cause of nosocomial Gram-negative bacillary infections (after *E. coli.*).

The results of this study indicated the high prevalence of *Enterobacter* species resistant to the majority of assessed antibiotics. In addition, prevalence rates of ESBL and carbapenemases producing *Enterobacter* species and MDR strains were high in Khartoum- Sudan.

Finally, *Enterobacter* species was relatively high in Sudan, and it seems that carbapenems cannot be considered the best drugs of choice for the treatment of MDR and ESBL-producing *Enterobacter* species.

Amikacin is the best drug choice to treat *Enterobacter* infection.

Recommendations

We suggest the management of antibiotic prescription, launching and developing health education and infection control programs, continuous monitoring of drug resistance and evaluation of the therapeutic efficacy of new antimicrobial agents. To accurate identification of species and subspecies must be developed.

Conflicts of Interest:

The authors declare that they have no competing interests.

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