Identification of Carbapenem Resistance among Enterobacteriaceae Pathogens Isolated from Clinical Samples in Shendi locality

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Abstract

One of the most serious issues in medicine is the increasing resistance of Enterobacteriaceae to antimicrobial agent's especially broad-spectrum antibiotics such as Cephalosporins and Penicillins. This fact is associated with higher mortality and morbidity rates, prolonged hospital stays, and increased treatment-related costs. Carbapenems are the last line for the treatment of infection caused by bacteria resistance to a broad spectrum of antibiotics. This study aimed to detect the Distribution of Carbepanems Resistance in Enterobacteriaceae Pathogens Isolated from Clinical Samples in the Shendi locality. Cross-sectional study and a laboratory-based study was carried out on 63 isolates from different specimens, 63 types of pathogenic bacteria were isolated and identified using Gram stain, biochemical reactions, and testing for their susceptibility to Carbapenems antibiotics was performed for all Enterobacteriaceae isolates. The isolated Enterobacteriaceae comprising of 19.0(30.2%) *E. coli*, 17 (27.0%) *K. pneumoniae*, 13(20.6%) *P. vulgaris*, 5(7.9%) *P. mirabilis*, 1(1.6%) *Enterobacter species*, 3(4.8%) *C. freundii* and 5(7.9%) M. morganii. Carbapenem (Imipenem and meropenem) susceptibility testing showed that 36.0% of Enterobacteriaceae isolates were Carbapenem resistant. The study revealed the low resistance pattern of Enterobacteriaceae to Carbapenems. Despite that *E. coli* (19.0, 30.0%) was the most isolated organism, *K. pneumoniae* (7.0, 11.0%) was the most resistant isolate to Carbapenems. The urine sample had the highest degree of resistance. There was a correlation between patients with chronic diseases such as cancer and the resistance level to Carbapenems, while there was no statistical significance between the period of antibiotics usage and the degree of resistance.

Keywords: *Antibiotic resistance, carbapenemases, Enterobacteriaceae, β-lactamase, Shendi, Sudan.*

Introduction

The Enterobacteriaceae is a large family of gram-negative rods found primarily in the colon of humans and other animals, many as part of the normal flora [1]. The family includes many genera (*Escherichia*, *Shigella*, *Salmonella*, *Enterobacter*, *Klebsiella*, *Serratia*, Proteus, and others [2]. All members of the Enterobacteriaceae, being gram-negative, contain endotoxin in their cell walls. In addition, several exotoxins are produced (e.g., *E. coli* and *Vibrio cholera* secrete exotoxins, called enterotoxins, that activate adenylate cyclase within the cells of the small intestine, causing diarrhea) [1]. Enterobacteriaceae are inhabitants of the intestinal flora and are among the most common human pathogens, causing infections such as cystitis and pyelonephritis with fever, septicemia, pneumonia, and peritonitis, meningitis, and device-

associated infections. Enterobacteriaceae are the source of community- and hospital-acquired infections. They have the propensity to spread easily between humans (hand carriage, contaminated food, and water) [3]. Carbapenems play a critically important role in our antibiotic armamentarium. Of the many hundreds of different β-lactams, carbapenems possess the broadest spectrum of activity and greatest potency against Gram-positive and Gram-negative bacteria. As a result, they are often used as "last-line agents" or "antibiotics of last resort" when patients with infections become gravely ill or are suspected of harboring resistant bacteria [4]. The term "carbapenem" is defined as the 4:5 fused ring lactam of penicillins with a double bond between C-2 and C-3 but with the substitution of carbon for sulfur at C-1. The hydroxyethyl side chain of thienamycin is a radical departure from the structure of conventional penicillins and cephalosporins, all of which have an acylamino substituent on the β-lactam ring; the stereochemistry of this hydroxyethyl side chain is a key attribute of carbapenems and is important for activity [5].Carbapenems demonstrate an overall broader antimicrobial spectrum in vitro than the available penicillins, cephalosporins, and _-lactam/_-lactamase inhibitor combinations [6].In general, imipenem, panipenem, and doripenem are potent antibiotics against Gram-positive bacteria, Meropenem, biapenem, ertapenem, and doripenem are slightly more effective against Gram-negative organisms [6].Carbapenem-resistant strains have emerged among species belonging to the Enterobacteriaceae family [7].Carbapenem-resistant Enterobacteriaceae (CRE) is rapidly spreading worldwide [8].Several outbreaks caused by carbapenem-resistant Enterobacteriaceae (CRE) have been recorded in health care facilities around the world[9]. And in some places, CRE has become endemic [10]. Carbapenemase-producing Enterobacteriaceae (CPE) contribute significantly to the global public health threat of antimicrobial resistance [11].

Carbapenemases increasingly have been reported in Enterobacteriaceae in the past 10 years. Klebsiella pneumonia carbapenemases have been reported in the United States and then worldwide [3].

Materials and methods

Study design:

This is a cross-sectional descriptive study that aimed to determine the Distribution of Carbepanems Resistance among Enterobacteriaceae Bacteria pathogens Isolated from Clinical Samples in the Shendi locality in the period from June to December 2021.

Study area:

This study was conducted at Shendi city which is a town in northern Sudan on the east bank of the river Nile 150km northeast of Khartoum. The area is inhabited by the gaaleen tribe.

Study duration:

This study was done from June to December 2021.

Study population:

Patients who are infected by Enterobacteriaceae bacteria live in the Shendi locality.

Sample size:

A total of sixty-three different clinical samples such as urine (40) wound swabs (13) and sputum (10) were collected from male and female patients.

Data collection tools:

The primary data was collected by using a structured questionnaire for collecting information on age, sex, use of antibiotics for a long period, history of chronic diseases, and use of antibiotics without a doctor's prescription.

Statistical analysis:

Data will be analyzed Data collected in this study will be analyzed using SPSS (version16).

Ethical considerations:

Samples collection will be explained to participants undergoing the test. All participants were informed about the research objectives and procedures during the interview period. Written valid consent will be obtained from all participants. All results will be with high privacy and confidentiality.

Sample collection:

Samples will be collected by suitable procedures depending on the site of infection, then they will be transferred as soon as possible to the microbiology laboratory to be cultured in suitable culture media and further investigations will be done.

Sample processing:

Clinical samples were collected from patients. Specimens, such as sputum and wound swabs were cultured in blood and MacConkey agar while urine was cultured in CLED media. Indirect Gram stain was done and Gram-negative rods were selected for further identification and testing, Subcultures for Gram-negative bacilli were done on blood agar, MacConkey agar, and CLED to ensure purity and incubated aerobically at 37 C for 24 hours. Then standard biochemical tests were performed including Oxidase tests, KIA, indole test, urease production

test, citrate utilization test, and motility tests.

Results

A study included (63) samples collected from (24) males and (39) females. In this study, the participant's ages ranged from (9) to (82) years, divided into 3 categories (Table1). With mean age (50) ± standard deviation. The study included three types of samples most of them were urine samples (40) and the other was wound swabs (13) and sputum (10) (Table2). The most abundant isolated organism in the urine sample was *E. coli* (13.0, 32.5%) and *K. pneumonia* (12.0, 30.0%), the swab sample was *P. Vulgaris* (4.0, 30.8%) and sputum sample was *K. pneumonia* (4.0,40.0%) (Table3), and Statistical analysis of antimicrobial susceptibility pattern of isolated bacteria against Carbapenems (Table4). The highest frequency of Carbapenems resistance was detected in urine (Table 5). About (37, 58.7%) of 63 patients had chronic diseases. The correlation between chronic diseases and Carbapenems resistance was significant, especially in cancer patients (Table6). The study revealed that there was no relation between the use of antibiotics for a long time and the increase in the degree of resistance (Table7).

Table 1: The distribution of study population according to age.

Age group	No	Percent %
Less than 30 years	19	30.2%
31-60 years	22	34.9%
More than 60 years	22	34.9%
Total	63	100%

Table 2: The distribution of samples according to type.

Age group	No	Percent %
Urine	40	63.5%
Swab	13	20.6%
Sputum	10	15.9%
Total	63	100%

Table 3: The bacterial strains isolated from samples

Sample	Bacteria						
	E. coli	*K. pneumoniae*	*C. freundii*	*Enterobacter*	*P. vulgaris*	*P. mirabilis*	*M. morganii*
Urine	13 (32.5%)	12 (30.9%)	1 (2.5%)	0 (0.0%)	8 (20.0)	3 (7.5%)	3 (7.5%)
Wound	4 (30.8%)	1 (7.68%)	1 (7.68%)	1 (1.5%)	4 (30.8%)	1 (7.68%)	1 (7.68%)
Sputum	2 (20.0%)	4 (40.0%)	1 (10.0%)	0 (0.0%)	1 (10.0%)	1 (10.0%)	1 (10.0%)
Total	19 (30.0%)	17(27.0%)	3 (4.5) %	1 (1.5%)	13 (20.0%)	5 (7.5%)	5 (7.5%)

Table 4: The antibiotics susceptibility pattern of isolated bacteria against carbapenems

Bacterial strains	No		Imipenenm	P.value		Meropenm	P. value
E. coli	19	S	15.0(78.9%)	0.994	S	13.0(68.4%)	0.846
		R	04.0(21.1%)		R	06.0(31.6%)	
K. pneumonniae	17	S	09.0(52.9%)	0.222	S	11.0(64.7%)	0.846
		R	08.0(47.1%)		R	06.0(35.3%	
C. freundii	3	S	02.0(66.7%)	1.000	S	02.0(66.7%)	1.000
		R	01.0(33.3%)		R	01.0(33.3%)	
Enterobacter	1	S	01.0(100%)	0.667	S	01.0(100%)	0.667
		R	00.0(0.00%)		R	00.0(0.00%)	
P. vulgaris	13	S	07.0(51.8%)	0.359	S	07.0(51.8%)	0.393
		R	06.0(48.2)		R	06.0(48.2)	
P. mirabilis	5	S	02.0(40.0%)	0.179	S	03.0(60.0%)	0.314
		R	03.0(60.0%)		R	02.0(40.0%)	
M. morganii	5	S	04.0(80.0%)	0.121	S	05.0(100. %)	0.121
		R	01.0(20.0%)		R	00.0(0.00%)	
Total	63	S	40.0(63.5%)		S	42.0(66.7)	
		R	23.0(36.5%)		R	21.0(33.3)	

Table 5: The distribution of samples according to type and carpabenems resistance.

Sample	No	Frequency	Imipenenm		P. Value	Meropenm		P. Value
Urine	40	63.5%	S	19.0(47.5%)	0.001	S	20.0(50%)	0.001
			R	21.0(52.5%)		R	20.0(50%)	
Swab	13	20.6%	S	11.0(84.6%)	0.888	S	12.0(92.3%)	0.888
			R	02.0(15.4%)		R	01.0(7.7%)	
Sputum	10	15.9%	S	10.0(100. %)	1.000	S	10.0(100%)	1.000
			R	00.0(00.0%)		R	00.0(00.0%)	

Table-6: The correlation between carbepanems among patient with chronic diseases.

Chronic diseases	Frequency	Imipenenm		P. Value	Meropenm	P. Value
No	26(41.3%)	S	23 (88.5%)	0.888	23 (88.5%)	0.888
		R	3 (11.5%)		3 (11.5%)	
D.M	12(19.0%)	S	11 (91.7%)	0.755	11 (91.7%)	0.755
		R	1 (8.3%)		1 (8.3%)	
Cancer	25(39.7%)	S	6 (24.0%)	0.001	8 (32.0%)	0.001
		R	19(76.0%)		17(68.0%)	

Table 7: The relation between uses of antibiotics for long period with carbapenems resistance

Use of antibiotics for long period	Imipenenm		P. Value	Meropenm		P. Value
Yes	S	25.0(64.1%)	1.000	S	27.0(69.2%)	0.595
	R	14.0(35.9%)		R	12.0(30.8%)	
NO	S	15.0(62.5%)	0.554	S	15.0(62.5%)	0.389
	R	09.0(37.5%)		R	09.0(37.5%)	

Discussion

This study done to detect the present of carpabenems resistance *Enterobacteriaceae*, 63 samples collected from males 24.0(28.0%) and females 39.0(62.0%) with different age group with mean (50.0) years ± SD (20.5), from different samples urine (40, 65.5%), swab (13, 20.6%) and sputum (10, 15.9%).In current study include, 40 urine samples, and the most common isolated organism from these samples were *E.coli* 13 (32.5%), *K. pneumonniae* 12 (30.0%), and the least isolated *C. furndii* 1 (2.5%), The present study demonstrates that antibiotic resistance for Imipenenm was 21 (52.5%) and for Meropenem was 20 (50.%), which means most patients with UTI have a high degree of carbapenems resistance, these findings were nearly in agreement with a study done by Elshamy and his colleagues who collected (256) urine samples, they found that a 212 / 256 (83%) were *Enterobacteriaceae*) from which 65/112 (25.4%) were Carbapenem resistance[12]. But were in disagreement with the study was done by Zavala and her colleagues collecting 296 urine samples,

most of isolated is *E. coli* and *K. pneumonniae, they* show no resistance against carbapenems [13]. These differences may be due to differences in Geographical area and environmental factors. Regarding swabs samples, the most isolated organisms were E. *coli* 4 (30.8%) and *P. vulgaris* 4 (30.8%), showing a very low degree of resistance against Imipenenm 2 (15.4%) and Meropenem 1(7.7%), that disagree with a study done by Tamma and his colleagues they collect wound swabs showed that Patients colonized with carbapenemase-producing CRE were more likely than those colonized with non-carbapenemase-producing CRE to develop CRE infections during their hospitalizations [14]. This difference may be due to the differences in sample size or the performed techniques. Considering sputum samples, there was no resistance to Imipenenm and Meropenem, this result is different from a study done by Adesanya and his colleagues who collect (13) sputum samples from which 4/13 (30.8) samples are Carpabenems resistant [15]. This difference may be due to differences in Geographical

area and environmental factors, considering the chronic diseases, there was a strong relationship between antibiotic resistance for Imipenem and Meropenem with a chronic disease like cancer as the *P. value* was (0.001), compared with those who have not any chronic diseases, this result agrees with a study done by Matthew. They found that patients with heart diseases or liver failure or malignancy had been infected by CRE more than CSE, which may be due to the low immunity in this group of patients [16]. The study illustrated that there were no statistically significant differences between long-term use of antibiotics and Carbepanems resistances as the *P. value* was (0.389), which disagrees with a study done byLlor and his colleagues that revealed Antibiotic overuse increases the development of drug-resistant bacteria [17].

Conclusion

The study revealed that there was a low resistance pattern of *Enterobacteriaceae* to Carbapenems (36.0%). Despite that *E. coli* was the most isolated organism, *K.pneumonniae* was the most resistant isolate to Carbapenems. Isolates from the urine sample had the highest degree of resistance. There was a significant correlation between patients with chronic diseases such as cancer and the resistance level to Carbapenems; while there was no statistical significance between the period of antibiotics usage and the degree of resistance.

Recommendation

1. Larger sample size should be tested to cover a wider range of isolates.
2. Specific methods for detection of Carbapenems resistance bacteria isolates should be used routinely such as the detection of carbapenemase enzyme production for the resistant isolates was performed using a modified Hodge test.
3. More specific tests include PCR for the detection of Carbapenems resistance genes.
4. A proper control strategy to control the spread of the resistance isolates and the use of antibiotics.

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Conflict of Interest

The author has affirmed that there are no conflicting interests.

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