



DETECTION OF CARBAPENEM RESISTANT BACTERIA GRAM NEGATIVE IN ENVIRONMENT WARDS AND UNITS HOSPITALS BASRAH, IRAQ.

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Abstract:

Several studies stated that hospital environment is considered as a reservoir of carbapenem resistant bacteria that contaminate and spread of these bacteria to resident patients. Infection with these bacteria associated with high mortality and morbidity. Two-hundred twenty-two swab samples were taken from different sites including wards and units of four teaching hospitals in Basrah city-Iraq and cultured on chromogenic medium HiCrome resist carba agar plates (Hi Media) for detection and screening of carbapenem resistant bacteria. Bacterial isolates were directly identified through colour differentiation on this medium. One-hundred and eighty-nine isolates out of two-hundred swab samples were detected as carbapenemase producers. Carbapenemase producing bacteria belonging to *Klebsiella pneumoniae* (61 isolates), *Escherichia coli* (37 isolates), *Pseudomonas aeruginosa* (19 isolates), *Acinetobacter baumannii* (4 isolates) and unidentified bacteria (68 isolates). Antibiotic susceptibility testing (Disc diffusion method) were also done for the isolates against four carbapenem antibiotics: imipenem, meropenem, ertapenem and doripenem to determine the level of susceptibility. The results were obtained as follows: Thirty isolates out of one-hundred and eighty-nine carbapenem producers showed resistance to most carbapenem groups. to four carbapenem antibiotics tested. Isolate Antibiotic susceptibility testing were determined. The results showed that 100% of the isolates were resistant to the antibiotic imipenem (30 isolates), and 26 (86.66%) isolates were resistant to meropenem. 17 (56.66%) ertapenem,11(36.66%) doripenem.

Keywords: Carbapenem, Multidrug resistant, hospitals environment

INTRODUCTION

Today, the world faces many challenges in the field of public health, due to the increasing numbers of infections and deaths caused by multidrug-resistant bacteria (MDR) to antibiotics pose a threat to global health and a cause of growing concern WHO (2021).ESKAPE is an acronym for the group of six bacteria that consists of Gram-positive bacteria, consisting of *Enterococcus faecium* and *Staphylococcus*, and Gram-negative Gram Stain *aeruginosa* *Pseudomonas*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Enterobacter* species (WHO, 2017). The aforementioned bacteria include the main causes of nosocomial infections life-threatening infections that lead to infection among critically ill and immunocompromised individuals (Pendleton *et al.*, 2013).

Carbapenem antibiotics are of the most effective antimicrobials in treating infections caused by multi-drug resistant bacteria. and is most effective against Gram positive and Gram-negative bacteria, usually prescribed as the last resort for the treatment of

severe life-threatening infections caused by MDR (Aurilio *et al.*, 2022).

There are three components to the hospital environment: the building or space used to provide medical services to patients, the equipment used to assist patients, and personnel including staff, patients, and guests. The patient environment is described as the immediate space around the patient that the patient or staff may touch when providing health care (Suleyman *et al.*,2018). Nosocomial infections are one of the leading causes of morbidity and mortality worldwide. Due to the increase in bacterial resistance, it is difficult to find the appropriate treatment (Tacconelli *et al.*, 2014). In previous studies, they indicated that hospital wards that are generally affected by health care-related infections (HAIs) are surgery, general medicine, pediatrics, intensive care, obstetrics and gynecology wards. burns unit, fractures and bruises unit (Chaibdraa & Bentakouk, 2008).

The hospital environment has a major role in the transmission of MDRGN-negative multi-resistant bacteria, and that infections with these resistant



bacterial species are associated with high mortality rates in recent years. (Protano, 2019) indicated that infections associated with medical units constitute a serious public health problem and contribute to an increase in death rates and hospital stays, as well as an increase in antimicrobial resistance. According to (Kotwani *et al.*, 2021; Song *et al.*, 2016), the hospital environment promotes biofilm growth in the presence of suboptimal concentrations of antibiotics by enhancing bacterial interaction and multiplying the proliferation of resistance genes (Weingarten *et al.*, 2018).

RESEARCH AIMS

Isolation and identification of Carbapenem-resistant Gram-negative bacteria from the environment of hospitals in Basra city.

METHODS:

Sample collection:

Two hundreds (200) samples were collected from the environment surrounding patients who had been hospitalized for more than a week, and from the different wards and departments of the four educational hospitals in Basrah, various environmental sources, including the intensive care unit, the burns center, the obstetrics and gynecology ward, the surgery and fractures ward, the internal ward, and Dialysis center, children's ward, nervous system ward, cardiopulmonary resuscitation, emergency ward), distributed as follows (Bed, Pillow, Wall, and Floor, Clothes worker, Patient clothes, Doors and Door handles, Bathroom Sinks, and Medical devices and Curtains). from (11/8/2021 AD) to (6/20/2022 AD) by means of cotton swabs containing transport media swabs, thus ensuring that the isolates remain active and transferred To the research laboratory as soon as possible (microbiology laboratory) shortly after collecting it and keeping it in the refrigerator at a temperature of 4°C. The samples were then grown on MacConkey agar medium.

Then, they were grown on HiCrom CarbaResist Agar (M2099) diagnostic medium for isolating and diagnosing Carbapenem-resistant bacteria. conventional, depending on the phenotypic and microscopic characteristics.

Isolation and identification of carbapenem-resistant isolates

The used HiCromTM Carba Resist a medium for detection and screening of carbapenem resistant bacteria. Bacterial isolates were directly identified through colour differentiation on this medium and confirmed based on the manufacturer's instructions..

Antimicrobial Susceptibility test (AST)

Antibiotic Susceptibility test was used, on Mueller-Hinton agar using modified Kirby-Bauer disc diffusion

method and interpreted based on the recommended breakpoints CLSI 2020.

RESULT AND DISCUSSION

The total number of samples was 200 samples, 78 samples (39%) showed negative growth for bacterial culture and 122 samples (61%) positive growth for bacterial culture. (189) isolates growing on HiCromTM Carba Resist agar media were obtained, (222) Isolate grown on medium MacConkey agar.

The results of the current study showed the possibility of isolating bacterial species resistant to carbapenems from the hospital environment on HiCromTM Carba Resist Agar medium and in abundance, and the highest number of isolates producing carbapenemase enzymes was from the intensive care and internal wards (39) Isolation followed by the burn center (26) isolation and then the emergency ward (24) isolation. From a total of (189) environmental samples distributed and developed on selective media distributed as follows in the emergency department, which is a vital department and is hardly devoid of patients lying in various cases, including simple and emergency In addition to dangerous and sensitive cases, 21 samples (24) of isolates were collected from different locations, and when planted on HiCromTM Carba Resist Agar medium, and 2 isolates belonged to *Klebsiella pneumoniae*, 12 isolates belonged to the bacteria *E. coli*, and 10 other isolates were diagnosed. As for the burns center in Al-Fayha Teaching Hospital, 18 samples (26) isolates were collected from the facilities of this center, 11 belonged to *Pseudomonas aeruginosa*, 2 isolates of *E. coli*, 3 isolates of *Klebsiella pneumoniae* and 10 other isolates. As for cardiopulmonary resuscitation, (16) samples were collected. And 9 isolates were one of the bacteria *E.coli* and 8 others isolates. In the intensive care unit ICU out of 41 samples were 39 isolates, as 14 isolates belonged to *Klebsiella pneumoniae* and 10 isolates belonged to *Escherichia coli* and 11 of the other isolates and 4 isolates belonged to *A. baumannii*.

As for the Obstetrics and Gynecology Unit, it collected 13 samples and 12 isolates, 7 isolates of which belonged to *K. pneumoniae*, 3 other isolates and 2 isolates from *E.coli* were distributed. As for the surgery and fractures ward, 24 samples were collected some of them grew and others did not appear on them, only 9 isolates were growing on the medium Carba Resist Agar HiCromTM for patients who had been hospitalized for more than a week 3 isolates belonged to *E. coli*, 3 isolates belonged to *Klebsiella pneumoniae*, and 3 other isolates. The atrium Esoteric Nervous system 16 samples and 14 isolates, 12 of which were *K. pneumoniae* isolates and 2 *E.coli* isolates.

As for the internal unit, it collected (36) samples of 39 isolates, and it was 5 isolates of *Escherichia coli*, 8

Pseudomonas aeruginosa, 11 *Klebsiella pneumoniae* and 15 other isolates. This is consistent with what was stated by the researcher (Almudhafar& Al-Hamdani ,2022), where 16 environmental isolates were collected from the wastewater of Al-Fayha Teaching Hospital, 4 of which belonged to *Enterobacter cloacae*, 4 isolates belonging to *Escherichia coli*, 2 *Acinetobacter*, 4 isolates from *EnterobactercloacaSSpdissolvensa* , 1 *Raoultella ornithinolytica*, and 1. *Enterobacter aerogenes*. As for the dialysis center, 8 samples and 7 isolates were collected. 4 isolates belonged to *K. pneumoniae* and 3 other isolates. As for (the children's section) Pediatrics, there were 7 samples and 10 isolates, 5 isolates of which belonged to *K. pneumoniae*, 5 other undiagnosed isolates, as shown in Table (1). And in previous studies conducted by Lalami *et al.*, (2016) in hospitals in the city of Fez in Morocco for environmental surfaces, who found that the dominance of bacterial isolates was distributed as follows and according to the lobbies studied, as it reached the highest percentage in the emergency department (19%), followed by the central unit (17%), neonatal unit (16%), kitchen (14%), fractures and intensive care units (9%), surgery, heart disease and digestive system units (8%), A study Mbololona *et al.*, (2020) dealt with bacteria with multiple antibiotic

resistance in the hospital environment. The study included four departments in hospitals, as the intensive care unit was the most contaminated unit with bacterial species, 14 of which belonged to Staphylococci, and one isolate belonged to enterobacteria *Raoultella terrigena*, and one isolate (*A.baumannii*).As for the surgery unit, it consisted of 10 isolates belonging to Staphylococci, and the negative bacteria included one isolate belonging to the family of Enterococcus (*C. freundii*), and two isolates belonging to non-fermenting bacteria (*A.baumannii*). In emergencies, 12 samples were collected, 9 isolates belonging to Staphylococci and growing on the selective medium Chrom ID, while enterobacteria were not present in a study Laxmi *et al.*, (2021) to investigate the microbial in the hospital environment in the city of Chevella in India. A total of 3492 environmental samples were taken from surface swabs from different locations of the hospital. They found that the highest number of bacteria was obtained from the general surgery ward (677), followed by the orthopedic surgery ward (585), and that the majority of the isolates belonged to *Bacillus* spp. and Coagulase -negative Staphylococci. Negative for blood clotting enzyme and less bacteria is *P. aeruginosa*.

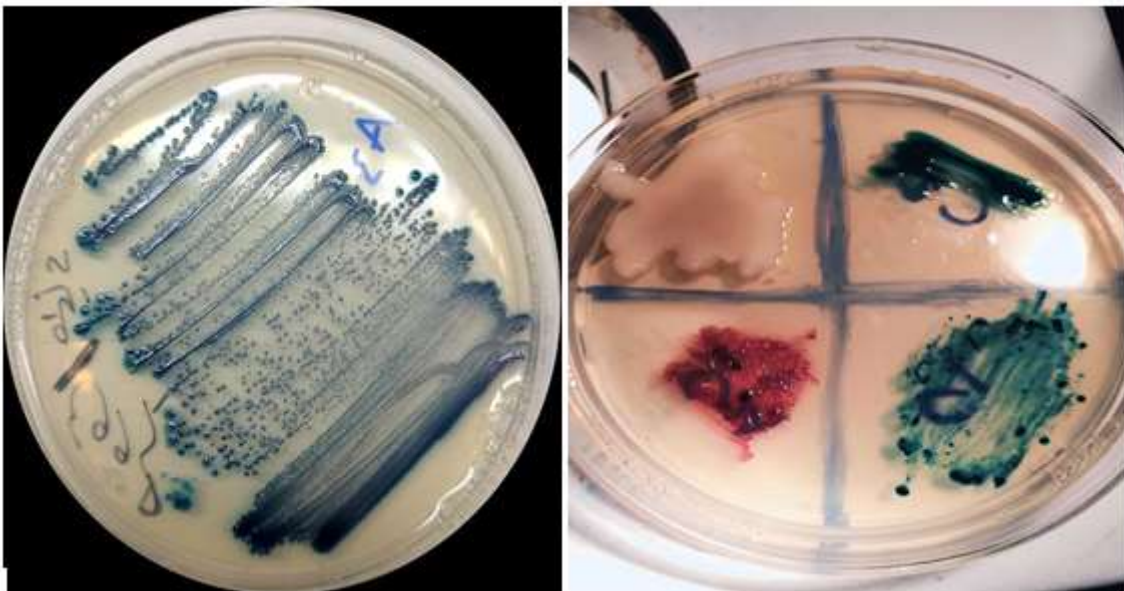


Figure No. (1) Cultures grown on HiCrom™ Carba Resist Agar medium

Table No. (1) distribution samples and isolates and the percentage of isolates from the departments of teaching hospitals in the center of Basra and isolated on the medium HiCrom Carba Resist Agar .

Place of Samples	NO. of samples	NO. of strains	Percentage%
Emergency ward(ER)	21	24	12.69%
Burns unit	18	26	13.75%
Cardiopulmonary resuscitation	16	9	4.76%
Intensive Care Unit(ICU)	41	39	20.63%



Obstetrics and Gynecology wards	13	12	6.34%
Surgery ward	24	9	4.76%
Nervous system atrium Esoteric	16	14	7.40%
Interior ward	36	39	20.63%
Dialysis center	8	7	3.70%
Pediatrics	7	10	5.29%
Total	200	189	99.5%

Obviously, the isolation of carbapenem-resistant bacterial species on specialized culture medium HiCrom™ carba Resist agar by developing isolates from environmental sources of different hospitals, and isolating and diagnosing the developing bacterial species through color differentiation, which is a medium designed to diagnose and distinguish the types of intestinal bacteria carbapenemase producing enterobacteriaceae. It is a medium used to screen these species. *Pseudomonas aeruginosa* and *Acinetobacter baumannii* also appear on this medium because they produce Carbapenemase. (Pillai *et al.*, 2009) In this study, a total of 189 carbapenem-

resistant isolates were isolated from a total of 200 environmental swab samples from various The hospital departments under study are as shown in **Table (2)**. A study (Abass and ALHamdani 2017) carbapenem resistance bacteria isolated from clinical samples in Basrah Governorate was found to *E. coli* be the most prevalent 18 (29.50%), followed by *K.pneumoniae* (22.95), *Pseudomonas aeruginosa* (11.47%), six isolates (9.83%) for both *Proteus mirabilis*, *Acinetobacter baumannii*, *Burkholderia cepacia* and one isolate (1.63%) for both, *Citrobacter frundill*, *Enterobacter cloacae*, *Serratia fonticola*, *Serratia marcescens*.

Table (.2) Bacteria that produce carbapenemase enzymes

<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>	Others	<i>A. baumannii</i>
37 (19.57%)	61 (32.27%)	19 (10.05%)	68 (35.97%)	4 (%2.11)

Antibiotic susceptibility testing by disc diffusion test

The results of the antibiotic sensitivity using the disc diffusion test showed that the total number of Carbapenem-resistant isolates are 30 bacteria isolates, at a rate of (15.87)% of the total 189 isolates grown on HiCrom™ Carba Resist Agar medium as in Table (3). These isolates showed resistance to all types of carbapenem antibiotics. the results showed that all the environmental isolates were resistant to the

antibiotic Imipenem 30(100%), Meropenem 26(86.66%), Ertapenem 17(56.66%), Doripenem 11(36.66%), and the most resistant isolates belong to the bacteria *Pseudomonas aeruginosa*. the results of the current study are almost consistent with the findings of researchers Kim *et al.*, (2022) who they found that 17 isolates out of 20 were resistant to Imipenem (80%), while 12 isolates (60%) were meropenem, 19 isolates were Ertapenem (95%) Figure 2.

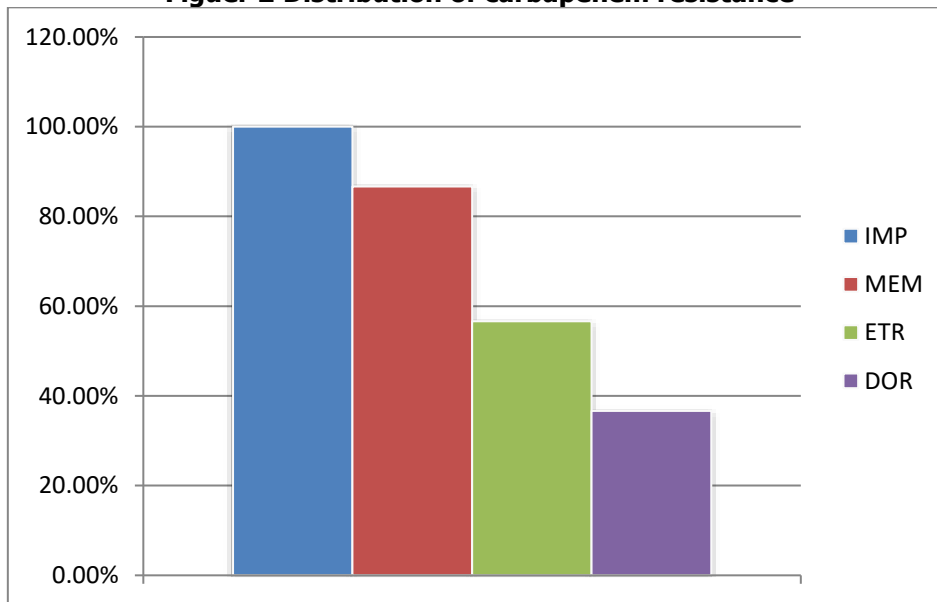
Table (3) Diameter of the inhibition zone for Carbapenem-resistant bacteria.

Symbol of isolates	Isolates	Isolate place	IM I	ME M	ET P	DO R
R3	<i>K. pneumoniae</i>	Icu	R	R	10	17
B6	<i>A. baumannii</i>	Icu	R	R	17	20
Y16	<i>K. pneumoniae</i>	Icu	R	14	16	20
Y19	<i>P. aeruginosa</i>	Inter	R	15	22	28
R1	Others	Icu	R	9	9	15
B9	Others	Icu	R	11	23	25
C10	Others	Sur	R	R	15	20
A2	Other s	Inter	R	10	17	22
Z5	<i>P. aeruginosa</i>	Burn	R	9	8	10
Z3	<i>P. aeruginosa</i>	Burn	R	9	8	10
Z4	<i>P. aeruginosa</i>	Burn	R	11	8	14
Z1	<i>P. aeruginosa</i>	Burn	R	R	R	10



O3	<i>K. pneumoniae</i>	ICU	R	10	R	20
N1	<i>P. aeruginosa</i>	Burn	R	10	8	14
N2	<i>P. aeruginosa</i>	Burn	R	10	8	12
Y6	<i>A. baumannii.</i>	ICU	R	8	10	12
Y7	<i>P. aeruginosa</i>	ICU	R	12	8	13
Y4	<i>A.baumannii.</i>	ICU	R	12	8	14
Y14	<i>P. aeruginosa</i>	Inter	R	9	8	13
Y28	Others	Inter	R	10	13	20
P1	<i>K. pneumonia e</i>	Inter	R	8	15	20
M2	<i>K. pneumoniae</i>	Obs	R	10	S	S
L3	<i>P. aeruginosa</i>	Burn	R	9	9	13
K1	<i>E. coli</i>	Burn	12	14	14	15
L5	<i>P. aeruginosa</i>	Burn	R	8	S	S
L2	<i>E. coli</i>	Burn	10	12	15	21
R2	Others	Icu	R	9	10	15
Y17	Others	Icu	R	15	16	21
N4	Others	Nerv	10	15	22	24
N5	<i>K. pneumoniae</i>	Nerv	10	15	20	22

Figuer 2 Distribution of carbapenem resistance



Distribution of total isolates and carbapenem-producing isolates within the environment of the four hospitals

Hospital environments present an opportunity for bacterial interaction and multiplication and infection of inpatients with hospital-acquired infections, also known as nosocomial infections, which carry a higher risk of death and prolonged hospitalization among patients as a large proportion of infections are caused

by bacteria that are resistant to antibiotics (Golle *et al.*, 2017).

The current study recorded out of a total of 200 samples, 189 carbapenemase-producing isolates were collected from teaching hospitals in the city of Basra, which were collected from different environmental isolation sources, including Bed 41 (21.69%), Pillow 33 (17.46%), Floor 34 (17.98%), Wall 7(3.70%),4.23%) Door 8 ,Clothes of worker11 (5.82%) ,Clothes of pateint14 (7.40%) , Curtain6(3.17%),,Sink17(%8.99)



Devices 18(9.52%)..can That contaminated non-living surfaces and health care providers participate in the transmission of hospital infections (Deshpande *et al.*, 2017), and it was found that the most carbapenem-resistant bacterial isolates were present in Al-Fayhaa Teaching Hospital (68 isolates (35.97%), followed by

Al-Sadr Teaching Hospital with 49 isolates and a rate of (%) 25.92), Basra Teaching Hospital 43 isolates (22.72%), while Mawani Teaching Hospital 29 isolates (15.34%) of the isolates producing carbapenemase enzymes, as can be seen from Table (4).

Table No. (4) Number of total isolates and their distribution within the four carbapenem-resistant hospitals

Hospitals	No. of Isolates	Hospitals	No. of Isolates
AL-sader N= (49)	Bed (10) Pillow (8) Floor (9) Wall (4) Door (4) Clothes of worker (2) Clothes of patient (4) Curtain (0) Sink (0) Devices (8)	AL-fayhaa N= (68)	Bed (20) Pillow (13) Floor (7) Wall (3) Door (2) Clothes of worker (5) Clothes of patient (3) Curtain (4) Sink (3) Devices (8)
AL-Basrah N= (43)	Bed (6) Pillow (7) Floor (7) Wall (0) Door (0) Clothes of worker (1) Clothes of patient (5) Curtain (1) Sink (14) Devices (2)	AL-Mawani N=(29)	Bed (5) Pillow (5) Floor (11) Wall (0) Door (2) Clothes of worker (3) Clothes of patient (2) Curtain (1) Sink (0) Devices (0)
92		97	
Total 189			

CONCLUSION: Our results revealed the prevalence of Carbapenem-resistant bacteria multi-drug resistance in the Basrah hospital environment from different sources in hospital units. Which poses an alarming threat to public health, which may cause the transmission of these CRB to inpatients or health care workers. This requires continuous sterilization and disinfection of all units and the establishment of regular monitoring programs to reduce and prevent pathogen.

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