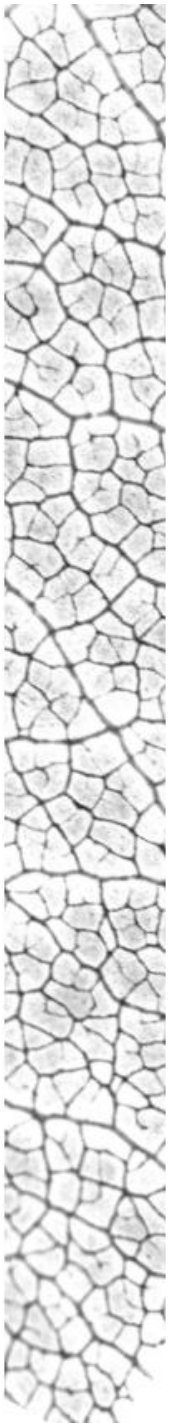

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RAPIDEC®CARBA NP-, CHROMID®CARBA agar- and MHT-based investigation of Carbapenem Resistance-Gram Negative (CRGN) bacteria isolated from wastewater in Basra city

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Abstract

Background: Carbapenem are a wide range of β-lactam antibiotics exploited as a final resort in the controlling of multidrug-resistant bacterial infections. Carbapenemase producing Gram negative bacteria may reach the wastewater via effluent of untreated hospital wastewater in addition to vital role of drug manufacturing companies that loaded their wastewater with such antibiotics leading to emergence of carbapenem resistance bacteria. The current study aims to investigate the carbapenemase producing Gram negative bacteria among wastewater using three different phenotypic methods. **Methodology:** During a winter period of 2018, 852 wastewater samples were collected from Basra city and cultured on MacConky agar and then confirm identification by API-20E. The Kirby-Bauer were used for screenings of carbapenem resistance and then the carbapenemase producing confirmed by three different phenotypic methods: MHT, RAPIDEC®.CARBA NP and CHROMID®.CARBA agar. **Results:** The results of bacterial isolation revealed positive culture for 742 (87.08%) of samples while 110 (12.92%) give no growth. Gram negative bacteria were recovered from 514/852 (60.32%) of samples and 228/852(26.76%) for Gram-positive bacteria. All 514 gram negative isolates were submitted for carbapenem producing using disc diffusion method for imipenem, meropenem and ertapenem. The results showed that only 38/514 (7.39%) of Gram negative isolates were resist at least one of the three used carbapenem antibiotics. 10/38 (26.31%) of Gram negative isolates were resist to meropenem, imipenem and ertapenem. 21/38 (55.26%) were resist to meropenem and imipenem while all isolates 38/38 (100%) were resist to meropenem. The 38 isolates of carbapenem producing Gram negative (CRGN) were 20/38 (52.631%) for *E. coli*, 11/38 (28.947%) for *K. pneumoniae*, 5/38 (13.157%) for *P. aeruginosa* and 2/38 (5.263%) for *P. mirabilis*. The results of three phenotypic assay to investigate the carbapenemase production showed that RAPIDEC®CARBA NP reveal 36/38 (94.74%), MHT reveal 31/38 (81.58%) and CHROMID®CARBA agar reveal 27/38 (71.05%) of isolates were carbapenemase producer. **Conclusion:** It is easily to conclude that, the carbapenemase producing Gram negative (CPGN) bacteria may reach the wastewater from hospital effluent and drug manufacturers. RAPIDEC®CARBA NP was rapid, accurate and suitable assay for detecting CPGN bacteria.

Keywords: Carbapenemase, wastewater, CHROMID®CARBA, RAPIDEC®CARBA NP, MHT

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INTRODUCTION

Carbapenem are a wide range of β-lactam antibiotics exploited as a best resort in the controlling of multidrug-resistant bacterial infections. In the last few decades, the advent of isolates with carbapenem-resistant has been a growing concern (Meletis, 2016). The World Health Organization (WHO) announced a list of the most harmful pathogens for human health in 2018. Top of the list are carbapenem-resistant *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and Enterobacteriaceae (Shrivastava, et al. 2018). Antimicrobial-resistant (AMR) bacteria are ever-present in rivers, oceans, lakes and sewer water (Hatosy, et al. 2015. Moore, et al. 2008. Marti, et al. Ash, et al. 2002. Pang, et al. 2015. Rosas, et al. 2015. Blaak, et al. 2014.Rodriguez-Mozaz, et al.

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2015). Water bodies implemented with biocides, antibiotics, heavy metals and other chemicals, that unsurprisingly handpicked for antimicrobial resistance within these waterborne microbial gene pools (Baquero, et al. 2008; Oyediran, et al, 2018).

Tenover, (2006). It is imperative to mention that numerous of antibiotics are manufactured by ecological microorganisms (Wikler, 2006). Conversely, genes of resistance, attained by harmful bacteria via Horzontal Gene Transfer (HGT) have been instigated as well in eco-friendly bacteria (Zarakolu, et al. 2015)., even though they can go forward far along on below robust antibiotic stress through the management of infections (Vrioni, et al. 2012. Papadimitriou-Olivgeris, et al. 2014). To comprehend the expansion of resistance, there was need to report the investigation of antibiotics and their resistance genes, not just in clinics but in natural non-clinical environments also (Baquero, et al. 2008). Carbapenem-resistant Gram-negative bacteria are chiefly studied as a cause of human infections, while reports concerning the existence of viable carbapenem-resistant bacterial populations outside medical institutions are globally rare. The current study was designed to evaluate the antibiotic resistance Gram negative isolated from Sage water in Basra. The research committed with supposition that natural environs could be occupied by multidrug resistant bacteria particularly to carbapenem antibiotic. Carbapenem resistance can be investigated phenotypically and genotypically. In recent times, numerous approaches have been developed to detect CPGN: (1) Modified Hodge test (MHT) is is a relatively easy and simple test to be performed in a laboratory. MHT has acceptable sensitivity and specificity for carbapenemase detection (Tenover, 2006. Wikler, 2006). (2) CHROMID®.CARBA agar has acceptable sensitivity (90.9 %) and the highest specificity (98.5 %) and also allowed for isolation of CPE within 18-20 h. CHROMID®.CARBA was found to be a rapid and accurate culture screening method for active CPE surveillance. CHROMID®.CARBA performed with high precision among the phenotypic strategies applied, giving early results and it is sensitive, specific, simple and cost-effective screening tests for detection of CRN isolates compared to the traditional MHT (Wikler, 2006. Zarakolu,et al. 2015, Vrioni, et al. 2012). (3) detection of carbapenem-hydrolysing process by monitoring the color alter of a pH indicator (the Carba NP check and its derivatives (Papadimitriou-Olivgeris, et al. 2014. Pires, et al. 2013). RAPIDEC®.CARBA NP is more specific and sensitive detecting any type of CPGN. it's a fast and easy-to-handle diagnostic take a look at for controlling the pass on of CPGN by detecting any sort (known or unknown) of carbapenemase activity. it could possibly finding its put as a first-line display of CPE in medical settings (Dortet, et al. 2014). The current study aims to detect the CRGN using three phenotypic method: RAPIDEC®CARBA NP, CHROMID®CARBA agar and MHT.

MATERIALS AND METHODS

Sampling

During a winter period of 2018, 852 wastewater samples were collected from Basra city, south of Iraq, and directly cultured on MacConkey agar (Himedia/India) and then, isolates were purified and identified by API 20E (Biomerieux/France).

Antimicrobial-susceptibility Assay

KirbyBauer disc-diffusion approach on Muller.Hinton Agar was used for susceptibility trying out for all recognized clinical isolates in keeping with the clinical and Laboratory standard Institute (CLSI18) pointers [20].

Carbapenemase-producing Detection by Disc Difussion

The recognition of carbapenemase-producing bacteria was achieved using the Kirby-Bauer disk-diffusion method using 10 µg ertapenem discs, 10 µg meperenem and 10 µg imipenem, on Muller-Hinton agar. Any strain having a decreased sensitivity to mropenem (inhibition zone <22 mm) was considered suspicious of being carbapenemase producing and was verified by other phenotypic tests as recommended by CLSI (Dortet, et al. 2015).

Carbapenemase-producing Detection by Modified Hodge Test (MHT):

A 0.5 MacFarland turbidity suspension of the E. coli strain E. coli ATCC 25922 was set and injected onto Muller Hinton Agar medium. The medium was dried and a disk of meropenem (10µg) was sited in the center of the test zone. Using a straightline swab, the test strain was distributed from the middle edge to the center and this was repeated with each of the test strains plus the control strains (positive and negative) in different directions. The samples were incubated overnight at 35°C +/- 2°C. The results were read after incubation; if bacterial growth in the form of flooding occured at the intersection of the E. coli 25922 inhibition zone with the line of the test strain (Dortet, et al. 2015).

Carbapenemase-producing Detection by CHROMID®.CARBA agar

The CHROMID®CARBA agar (Ref 414012) bi-plate comprises one half specific for blaOXA-like producing organisms while the other half is specific for blaVIM, blaNDM, blaKPC and blaIMP producing organisms.

Carbapenemase-producing Detection by RAPIDEC®.CARBA NP

The RAPIDEC®.CARBA NP is a ready-to-use strip and employs the principles of the Carba NP test, a novel phenotypic carbapenem hydrolysis test. It was achieved as advised by the manufacturer. A loopful (10 loop) of a bacterial colony was handpicked from overnight-incubated Mueller-Hinton agar plates and combined into API suspension medium(provided with kit); the bacterial

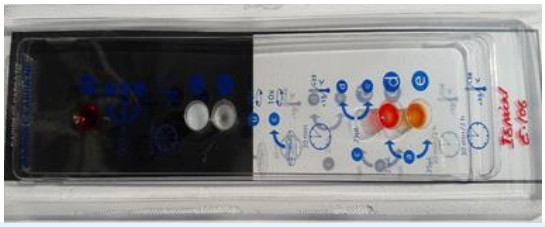

Fig. 1. RAPIDEC®.CARBA NP panel

Table 1. Distribution of bacterial isolates types among positive culture

Total No.=852	Growth Positive		No growth
	Gram Negative (GN)	Gram Positive (GP)	
NO.	514	228	110
%	60.32	26.76	12.92

Table 2. Distribution of carbapenem resistance among Gram negative isolates

Total No. of GN=514	Carbapenem	
	Resistant-CRGN	Sensitive-CRGN
NO.	38	476
%	7.39	92.61

CRGN=carbapenem resistant Gram negative

suspension was then putted to wells with check strip and incubated at 37°C. Optical studying of the test strip was finished wards 30 min and later 2 h, if essential. a +ve matched to a shade alter from red to yellow-orange, whereas a red color indicated a –ve results (1).

RESULTS

The results of bacterial isolation revealed positive culture for 742 (87.08%) of samples while 110 (12.92%) give no growth. Gram negative bacteria were recovered from 514/852 (60.32%) of samples and 228/852 (26.76%) for Gram positive bacteria (**Table 1**). All 514 gram negative isolates were submitted for carbapenem producing using disc diffusion method for imipenem, meropenem and ertapenem. The results showed that only 38/514 (7.39%) of Gram negative isolates were resist at least one of the three used carbapenem antibiotics (**Table 2**). 10/38 (26.31%) of Gram negative isolates were resist to meropenem, imipenem and ertapenem. 21/38 (55.26%) were resist to meropenem and imipenem while all isolates 38/38 (100%) were resist to meropenem (**Fig. 2**). The 38 isolates of carbapenem producing Gram negative (CRGN) were 20/38 (52.631%) for *E. coli*, 11/38 (28.947%) for *K. pneumoniae*, 5/38 (13.157%) for *P. aeruginosa* and 2/38 (5.263%) for *P. mirabilis* (**Table 3**).

Table 3. Distribution of carbapenem resistance among CRGN

bacteria	CRGN isolates			
	E. coli	*K. pneumonia*	*P. aeruginosa*	*P. mirabilis*
No.	20/38	11/38	5/38	2/38
Percentage	52.631%	28.947%	13.157%	5.263%

The results of three phenotypic assay (RAPIDEC®.CARBA NP, CHROMID®.CARBA agar- and MHT) to investigate the carbapenemase production were displayed in **Fig. 3**. The results showed that RAPIDEC®CARBA NP reveal 36/38 (94.74%), MHT reveal 31/38 (81.58%) and CHROMID®CARBA agar reveal 27/38 (71.05%) of isolates were carbapenemase producer.

DISCUSSION

The results showed the E. coli as an abundant gram negative bacteria followed by K. pneumoniae in Basra wastewater. The same results were also documented by Mounas et al. (2019), whose found 6 different bacterial species in the observed samples with frequencies reach to 50 colonies, Bacillus, E. coli, Klebsiella, Candida, Staphylococci, and Hyphae fungi. The presence of bacterial resistance is clear regions where anti-

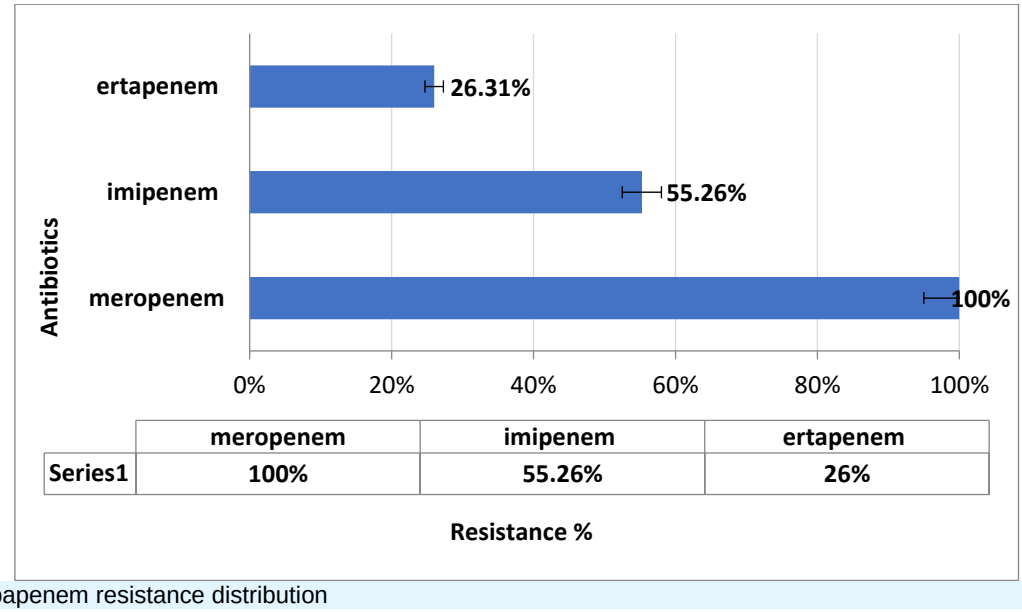

Fig. 2. Carbapenem resistance distribution

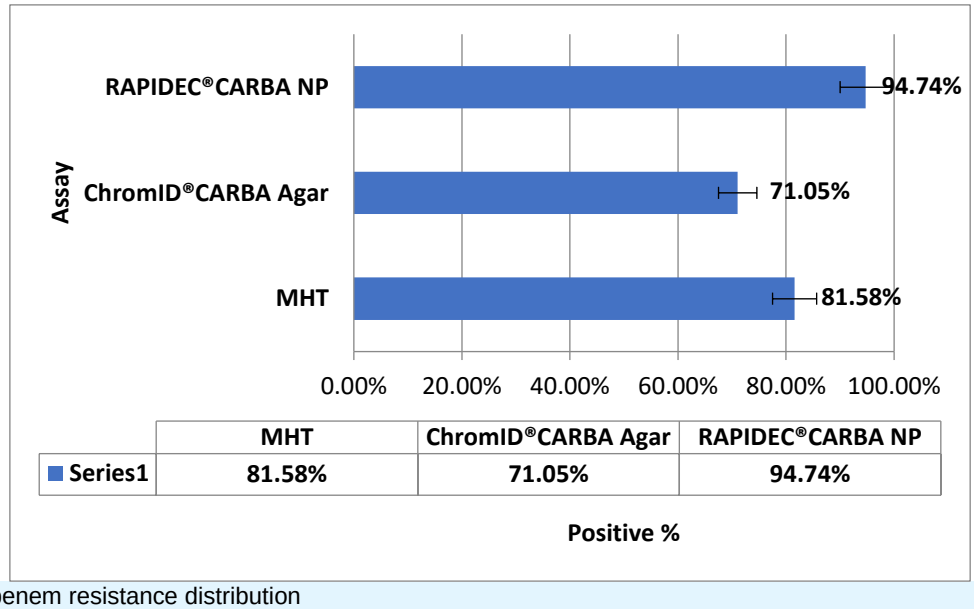

Fig. 3. Carbapenem resistance distribution

microbials are gigantically utilized, and antibiotic-resistant bacteria additionally progressively happen in aquatic environments. wastewater the board practice finances to the specific increase of antibiotic resistant bacteria and the occurrence of multi-drug resistant bacteria in aquatic environments. (Mounas, et al. 2019). The wastewater microbiome brings together bacteria of environmental, human and animal origins, many harboring antibiotic resistance genes (ARGs) (Manaia, et al. 2018). Wastewater treatment offices are among the fundamental wellsprings of anti-infection agents' discharge into the earth. The presence of anti-infection agents may underwrite the determination of antibiotic resistance genes (ARGs) and antibiotic resistant bacteria (ARB), which shade health risks to humans and animals (Rizzo, et al. 2013). *Escherichia coli* is copious in water frameworks and is a with respect to pool for AMR in these areas. A review of AMR *E. coli* in the Netherlands indicated 17.1% of ESBL E. coli secluded from river water and wastewater were reported as pathogenic, and of those pathogenic strains, approximately 84% exhibited resistance in up to three drug classes including beta-lactams, tetracyclines, and aminoglycosides (Rizzo, et al. 2013. Jang, et al. 2017).

section of multiresistant Enterobacteriaceae into nature thus into the evolved way of life is by means of waste water (Franz et al. 2015). microscopic organisms in the amphibian condition Some of these examinations that carbapenemases will follow these strides and reports have been distributed of carbapenemase-creating Enterobacteriaceae in the sea-going environment (Zurfluh, et al. 2013).

Carbapenemase producing Gram negative bacteria may be from hospital wastewater (Galler, et al. 2014).

Additionally, the drug manufacturing amenities ends in remarkable contamination of water sources with antimicrobial pharmaceuticals, which appears to be like to be related to the choice and dissemination of carbapenemase-producing pathogens (White, et al. 2016). Also, there is a risk of horizontal transfer of antibiotic resistance between clinical pathogens and environmental bacteria (Lübbert, et al. 2017). The emergence and speedy pass on of carbapenemase in Enterobacteriaceae and Pseudomonas species is changing into a primary inhabitants well being disaster worldwide, and is answerable for hefty collection of hospital-acquired and nosocomial infections (Diene, & Rolain, 2014). Concern the differences between results of three phenotypic methods: RAPIDEC®CARBA NP, CHROMID®CARBA agar and MHT may be easily explained. Incorrect finding of carbapenemase manufacturing was noticed by the MHT maybe on account of extended-spectrum β-lactamase (ESBL) manufacturing together with porin loss as reported before. scientific laboratories ought to be attentive to this fact, mainly in geographical areas the place ESBL-producing isolates are extremely predominant (Carvalhaes, et al. 2010). false +ve MHT almost certainly outcomes from low-level carbapenem hydrolysis by ESBLs, in particular these of the CTX-M variety (Girlich, & Nordmann2008).

CONCLUSION

It is easily to conclude that, the carbapenemase producing Gram negative (CPGN) bacteria may reach the wastewater from hospital effluent and drug manufacturers. RAPIDEC®.CARBA NP was rapid, accurate and suitable assay for detecting CPGN bacteria.

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