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Characterization of Carabapenem-resistant Enterobacteriaceae in Fresh vegetables

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Abstract

Carbapenem-resistant *Enterobacteriacea* (CRE) has become a growing food safety issue and an ongoing public-health problem of global dimensions. This research study determined the presence of CRE in fresh vegetables. Vegetables were purchased from the retailers and analyzed for *Enterobacteriacea*e in line with Standard guidelines. The antibiotic profile of the isolated *Enterobacteriaceae* was determined using Antibiotic Susceptibility Test (AST). The Modified Hodges test (MHT), Carbapenem inactivation method (CIM), Modified carbapenem inactivation (mCIM), and Ethylenediamine tetraacetic acid (EDTA) carbapenem inactivation (eCIM) were used screening for carbapenem resistance among Multidrug-resistant (MDR) Enterobacteriaceae. Forty-six *Enterobacteriaceae* were isolated in all. Among organisms isolated were *Citrobacter freundi*, *Enterobacter cloacae, Salmonella typhi, Klebsiella* sp, and *Escherichia coli*. AST of the isolates showed that two out of three third-generation antibiotics used had the highest resistance of 100%. Thirty-six (78.26%) were multidrug-resistant, eighteen were CRE using MHT but using CIM, and sixteen were CRE. Eighteen were CRE using the mCIM method, while 17 were using eCIM. Conclusion: This study shows that vegetables can be a source of carbapenem-resistant *Enterobacteriaceae*, and mCIM combined with eCIM is more sensitive in detecting CRE. Significance and Impact of Study: Ready-to-eat fresh Vegetables contain CRE.

Keywords: Vegetables; Modified Hodges test; Carbapenem inactivation method; Modified carbapenem inactivation; and EDTA carbapenem inactivation.

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Introduction

Vegetables and Fruits are rich sources of vitamins (B, C, K), minerals (calcium, potassium, and magnesium), and fiber for humans (Yahia et al.., 2019). Apart from the nutritional benefits, fruits and vegetables help to prevent different types of chronic illnesses such as heart diseases, cancer, and diabetes (Goodburn and Wallace, 2013; Septembre-Malaterre et al.., 2018).

However, besides their nutritional benefits, fresh vegetables and fruits can also be potential sources of various foodborne infections and disease outbreaks due to bacterial contamination as they are mainly eaten raw or slightly cooked (Callejon et al., 2015; Mir et al., 2018).

According to Nair and Vaz (2013), any *Enterobacteriaceae* that has resistance to one or more of the mentioned carbapenems: ertapenem, meropenem, imipenem, or doripenem and resistant to the

following third-generation cephalosporins: Ceftriaxone, cefotaxime, and ceftazidime are known as Carbapenem-resistant *Enterobacteriaceae* (CRE). CRE has a high mortality and morbidity rate. A 40%–50% death rate from CRE has been documented (Nair and Vaz, 2013).

CRE is primarily reported in samples of human origin (Singh-Moodley and Perovic, (2016); Lodise et al. (2017); Zhang et al. (2017), animals (Liu et al. (2017), health-center wastewater (Lamba et al. (2017), seafood product (Morrison and Rubin, (2015) and retail meat (Wang et al., 2017). However, studies focusing on CRE in fresh vegetables still need to be completed. Studies on CRE in Nigeria, though few, are concentrated in hospital settings (Olowo-okere *et al.,* 2020; Aminu et al., 2021). Hence, there is a crucial need to study the prevalence of CRE in fresh vegetables, especially fresh-to-eat vegetables in Nigeria.

Materials And Method

Fresh vegetables (Cabbage, Cucumber, Carrot, and Spring onions) were purchased from retailers between May to July 2021 and analyzed for *Enterobacteriacea*e using conventional biochemical methods (Cheesebrough, 2002). Antibiotic sensitivity test (AST) was carried out with the following antibiotics: Ceftazidime (CEF), Cefuroxime (CRX), Gentamicin (GEN), Cefixime (CXM), Ofloxacin (OFL), Augmentin (AUG), Nitrofurantin (NIT) and Ciprofloxacin (CPR) and the result was read using Clinical and Laboratory Standards Institute (CLSI) guideline, 2019. Modified Hodges Test (MHT), Carbapenem inactivation method (CIM), and modified carbapenem inactivation method (mCIM) were carried out on all isolates that were resistant to over one class of antibiotics.

Little adjustment was made to Aminu et al. (2021) descriptions of the modified Hodges test (MHT). *Escherichia coli* ATCC 259922 was used as a 0.5 McFarland dilution in 5ml of sterile water and streaked as a lawn on a Mueller Hinton agar (MHA) plate. The Petri dish's middle was filled with a meropenem susceptibility disk. The test organism was used to make a straight line from one edge of the disc to the edge of the plate. The dish was incubated for 18 - 24 hours at 37°C at night. MHT positive test shows a clover leaf-like indentation of the *Escherichia coli* 25922 growing along the test organism streak within the disc diffusion zone. A negative result shows no growth of known organisms along the test organism growth streak within the disc diffusion.

The carbapenem Inactivation Method (CIM) was carried out according to van de Zwaluw *et al.,* 2015. Streaked in 1 ml of Mueller Hinton broth was the test organism (MHB). Meropenem disc was submerged in the solution and heated to 37°C for two hours.The incubated meropenem disc was likewise placed on MHA and incubated for 18 hours at 37°C with a known CIM-positive bacterium. A negative result is indicated by a clear zone of inhibition by the test organism, while a positive result is denoted by neither.

Modified Carbapenem Inactivation Method and eCIM were performed according to Tsai et al., 2020 with little modification. A 1-µL loopful of test isolate was suspended in the 2-mL tube of nutrient broth and another 2-mL tube of nutrient broth supplemented with EDTA (Thermo Fisher Scientific, Carlsbad, CA, USA) at a final concentration of 5 mM (20 µL of 0.5 M EDTA in 2 mL of nutrient broth). Meropenem disk was placed in each tube and incubated at 35°C for four h ± 15 min. The disks were removed and placed on MHA plates on which 0.5 McFarland suspension of a carbapenem-susceptible *E. coli* ATCC 25922 strain has been streaked. Plates were incubated at 35 °C for 16 to 20 h. The isolate is mCIM positive if the zone size is 6 to 15 mm, intermediate for a zone size of 16-18 mm, and negative if the zone size is ≥19 mm. The isolate is eCIM positive if the zone size increases by ≥5 mm compared to the zone size observed for the mCIM and negative if the increase in zone size is < 4 mm (Sfeir *et al.,* 2019).

Results

Antimicrobial Susceptibility Test Of The Enterobacteriaceae Isolates

Forty-Six *Enterobacteriaceae* belonging to seven genera was isolated from the samples. They are *Citrobacter freundi*, *Enterobacter cloacae, Salmonella typhi, Klebsiella* sp*, Escherichia coli, Proteus mirabilis, Salmonella typhii*, and *Salmonella typhii*.

Table 1 shows the multidrug resistance activity of the isolated organisms. Thirty-six out of the forty-six organisms were resistant to more than two classes of antibiotics.

Table 1: Antibiotics Activity of the Isolated Organisms.

	CAZ	CTR	GEN	CXM	OFL	AUG	NIT	CPR	MULTIDRUG (≥2 CLASSES OF ANTIBIOTICS)
Klebsiella spp	R	R	S	R	S	R	R	S	YES
Citrobacter freundii	R	R	S	R	S	R	S	S	YES
Klebsiella spp	R	R	R	R	R	R	R	R	YES
Klebsiella spp	R	R	R	R	R	R	R	S	YES
Klebsiella spp	R	R	S	R	S	R	R	S	YES
Enterobacter cloacae	R	R	S	R	S	R	R	S	YES
Escherichia coli	R	R	S	R	S	R	S	S	NO
Proteus mirabilis	R	R	S	R	S	R	R	S	YES
Klebsiella spp	R	R	S	R	R	R	R	S	YES
Citrobacter freundii	R	R	R	R	R	R	R	R	YES

Enterobacter cloacae	R	R	S	R	S	R	S	S	NO
Klebsiella spp	R	R	S	R	S	R	R	S	YES
Klebsiella pneumonia	R	R	R	R	R	R	R	R	YES
Enterobacter cloacae	R	R	R	R	R	R	R	R	YES
Salmonella typhii	R	R	R	R	R	R	R	R	YES
Klebsiella	R	R	R	R	S	R	R	S	YES
Enterobacter cloacae	R	R	R	R	R	R	R	R	YES
Enterobacter cloacae	R	R	R	R	R	R	R	R	YES
Enterobacter cloacae	R	R	R	R	R	R	R	R	YES
Klebsiella spp	R	R	S	R	S	R	R	S	YES
Klebsiella spp	R	R	R	R	R	R	R	R	YES
Citrobacter freundii	R	R	R	R	R	R	R	R	YES
Enterobacter cloacae	R	R	S	R	S	R	R	S	YES
Salmonella typhii	R	R	S	R	S	R	R	S	YES
Enterobacter cloacae	R	R	S	R	S	R	R	S	YES
Citrobacter freundii	R	R	S	R	S	R	S	S	NO
Salmonella typhii	R	R	R	R	R	R	R	R	YES
Citrobacter spp	R	S	S	R	S	R	S	S	NO
Salmonella typhii	R	R	S	R	S	R	S	S	NO
Citrobacter freundii	R	R	R	R	R	R	R	R	YES
Citrobacter freundii	R	R	S	R	R	R	S	S	YES
Klebsiella spp	R	R	S	R	R	R	R	S	YES
Klebsiella spp	R	S	S	R	R	R	S	S	YES
Citrobacter freundii	R	S	S	R	R	S	S	S	NO
Citrobacter freundii	R	S	S	R	R	S	S	S	NO
Citrobacter spp	R	S	S	R	S	S	S	S	NO
Shigelia boydi	R	R	R	R	S	R	R	S	YES
Klebsiella spp	R	R	S	R	S	R	R	S	YES
Enterobacter cloacae	R	R	S	R	R	R	R	S	YES
Escherichia coli	R	R	R	R	R	R	R	R	YES
Enterobacter cloacae	R	R	R	R	R	R	S	S	YES

Citrobacter freundii	R	R	R	R	R	R	R	R	YES
Citrobacter freundii	R	R	S	R	R	R	R	S	YES
Klebsiella spp	R	R	S	R	R	S	S	S	NO
Klebsiella spp	R	S	S	R	S	R	S	S	NO
Escherichia coli	R	R	R	R	R	R	S	R	YES

R = Resistant

S = Sensitive

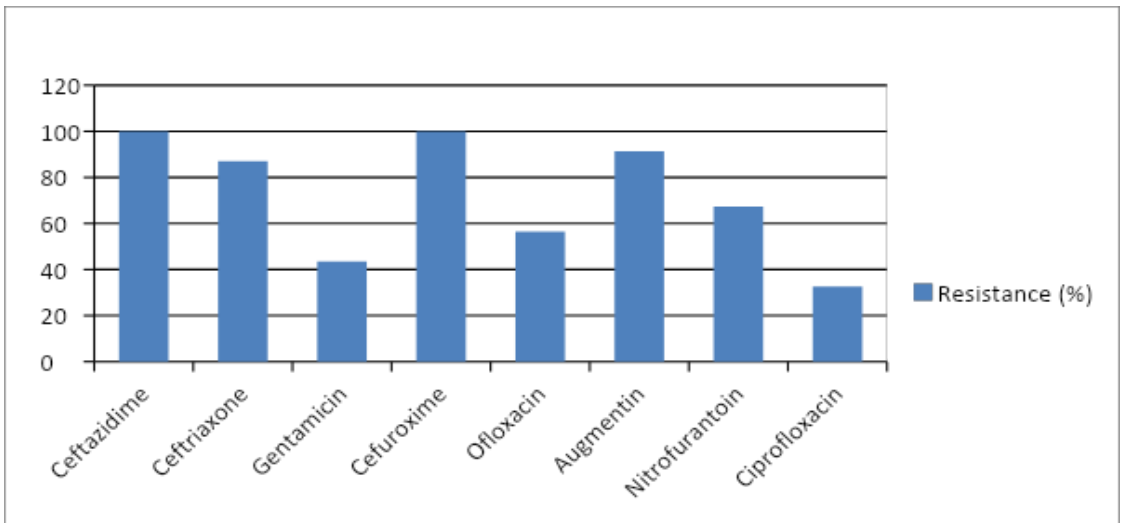

Figure 1: Antimicrobial susceptibility test of the *Enterobacteriaceae* isolates.

Figure 1 shows the percentage resistance of each antibiotic. Two (Ceftazidime and Cefixime) out of the three third-generation Cephalosporins had the highest resistance of 100%, followed by Augmentin at 91.3%, Ceftriaxone at 86.96%, Nitrofurantoin at 67.39%, Ofloxacin 56.52%, Gentamicin 43.48%, and Ciprofloxacin with the lowest resistance of 32.61%.

Phenotypic Characterization Of Multidrug-Resistant Strains

Table 2 shows the summary of the Modified Hodges test, Carbapenem inactivation test, Modified Carbapenem inactivation, and EDTA Carbapenem inactivation test for the thirty-six multidrug-resistant isolates.

TABLE 2: MHT, CIM, eCIM& mCIM OF MULTIDRUG RESISTANT *ENTEROBACTERIACEAE* ISOLATED FROM FOUR FRESH VEGETABLES (Cabbage, Cucumber, Carrot and Spring onions)

Isolate NO	Organisms	MHT	CIM	mCIM	Ecim
1	*Enterobacter cloacae*	+	+	+	+
2	Klebsiella spp	+	+	+	+
3	*Klebsiella* spp	+	+	+	+
4	Citrobacter freundi	-	-	-	-
5	*Klebsiella* spp	-	-	-	-
6	Klebsiella spp	+	+	+	+
7	*Escherichia coli*	-	-	-	-
8	*Proteus mirabilis*	-	-	-	-
9	*Klebsiella* spp	+	-	+	+

10	*Citrobacter freundii*	+	+	+	-
11	*Enterobacter cloacae*	-	-	-	-
12	*Klebsiella* spp	-	-	-	-
13	*Klebsiella pneumonia*	+	+	+	+
14	*Enterobacter cloacae*	+	+	+	+
15	*Salmonella typhi*	+	+	+	+
16	*Klebsiella* spp	+	+	+	+
17	Klebsiella spp	+	+	+	+
18	*Enterobacter cloacae*	-	-	-	-
19	Salmonella typhi	+	-	+	+
20	Enterobacter cloacae	-	-	-	-
21	*Klebsiella* spp	+	+	+	+
22	*Citrobacter freundii*	-	-	-	-
23	*Enterobacter cloacae*	+	+	+	+
24	*Salmonella typhi*	-	-	-	-
25	*Enterobacter cloacae*	+	+	+	+
26	*Citrobacter freundii*	-	-	-	-
27	*Salmonella typhi*	-	-	-	-
28	*Citrobacter freundii*	-	-	-	-
29	*Klebsiella* spp	+	+	+	+
30	*Citrobacter freundii*	-	-	-	-
31	*Citrobacter* sp	-	-	-	-
32	*Citrobacter* sp	-	-	-	-
33	*Shigalis boydii*	-	-	-	-
34	*Klebsiella* sp	+	+	+	+
35	*Enterobacter cloacae*	+	+	+	+
36	*Escherichia coli*	-	-	-	-

(+) means POSITIVE for carbapenemase production.

(-) means NEGATIVE for carbapenemase production

Discussion

Forty-six *Enterobacteriaceae* were isolated in all. Although *Klebsiella* was not identified at the specie level in this study, it was the most isolated bacterial specie (33%), which agrees with the report of Wang et al. (2018) and Adesanya and Igwe (2020), who both reported *K. pneumoniae* as the most prevalent organism isolated but contrary to the report of Al-kharousi *et al.* (2016) and Aminu et al. (2021) in which *E. coli* was the most dominant organism isolated from their work. The variation of this research with Al-kharousi et al. (2016) may be a result of seasonal variation at the time of purchase and difference in location since both studies were on fresh vegetables and fruits. Some other Gram-negative bacteria isolated include *Citrobacter* (26%), *Enterobacter* (21%), *Proteus* (2%), *Salmonella* (8%), and *Shigella* (2%), which suggests that various genera of bacteria should be monitored in the future.

Two (Ceftazidime and Cefixime) out of the three third-generation cephalosporins had the highest resistance of 100%, followed by Augmentin at 91.3%, Ceftriaxone at 86.96%, Nitrofurantoin 67.39%, Ofloxacin 56.52%, Gentamicin 43.48%, and Ciprofloxacin with the lowest resistance of 32.61%.

Thirty-six (78.3%) out of 46 isolated *Enterobacteriaceae* obtained in this study had resistance to two or more conventional classes of antibiotics and were regarded as multidrug-resistant (MDR) organisms. It was on the thirty-six MDRs that MHT was carried out. The prevalence of CRE was 50% (n = 18/36). Of these, 50% (n = 9) were *Klebsiella pneumonia*, 27.78% (n=5) were *Enterobacter cloacae,* 11.11% (n = 2) were *Citrobacter freundi***,** and

11.11% (n = 2) were *Salmonella typhi*. Fifty percent CRE obtained in this study is higher than 6.5% by Olowo-okere et al. (2020), 7.9% by Aminu *et al.* (2021), 22% reported by Adesanya and Igwe (2020), 27.4% by Olalekan *et al*. (2020) and 36.8% by Ogbolu et al. (2014). The differences might be due to the source and handling of the samples.

All the CRE isolates were resistant to all the three third-generation cephalosporins used in this study which is a 100% resistance rate and higher than the 44.2% resistant to third-generation cephalosporin reported by Olowo-okere et al. (2020). All eighteen CRE were positive for mCIM, seventeen were positive for eCIM, and sixteen were positive for CIM, implying 94.4% Metallo beta-lactamase and 5.5% non-Metallo beta-lactamase. This result agrees with the study of Tsai et al. (2020), who concluded that mCIM combined with eCIM is more sensitive in detecting CRE when compared with MHT.

Conclusion: This study shows that vegetables can be a source of carbapenem-resistant *Enterobacteriaceae*, and mCIM combined with eCIM is more sensitive in detecting CRE when compared with MHT alone.

Recommendation

The presence of CRE-producing organisms in the ready-to-eat vegetables analyzed is alarming and constitutes a food safety issue; therefore, measures need to be taken to ensure consumer health is monitored. Based on *Enterobacteriaceae's* multi-resistance to antibiotics, people should be enlightened on how best to consume vegetables in order to avoid foodborne illnesses. Also, research should not only be on the isolation of enteric bacteria like *E. coli* on vegetables from the markets, farms, and our environment, but more research on CRE needs to be carried out on ready-to-eat vegetables and fruits in Nigeria. Vegetables and fruits should be washed either with salt, warm water, or white vinegar to reduce microbial load before consumption.

Consent for publication: Not applicable

Competing interest: Not applicable.

Geolocation information: Abeokuta is the capital of Ogun State in Southwestern Nigeria, located on top of the east bank of the Ogun river, close to a group of stony outcrops in wooded savanna, 77 kilometers (48 mi) north of Lagos by railway or 130 kilometers (81 mi) by water. Latitude: 7° 09' 23.40" N, Longitude: 3° 20' 32.40" E.

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Abbreviations

Carbapenem-resistant *Enterobacteriacea* CRE

Multidrug resistant - MDR

Modified Hodges test - MHT

Carbapenem inactivation method - CIM Modified carbapenem inactivation

EDTA carbapenem inactivation - eCIM

Modified carbapenem inactivation - mCIM

References

Adesanya, O.A. and Igwe, H.A. (2020). Carbapenem-resistant *Enterobacteriaceae* (CRE) and gram-negative bacterial infections in south-west Nigeria: a retrospective epidemiological surveillance study. *AIMS Public Health*, 7 (4): 804–815. DOI: 10.3934/publichealth.2020062

Al-Kharousi, B.Z.S., Guizani, N., Al-Sadi, A.M., Al-Bulushi, I.M. & Shaharoona, B. (2016). Hiding in Fresh Fruits and Vegetables: Opportunistic Pathogens May Cross Geographical Barriers. IJM Volume 2016, Article ID 4292417, 14 pages http://dx.doi.org/10.1155/2016/4292417

Aminu, A., Daneji, I.M., Yusuf, M.A., Jalo, R.I., Tsiga-Ahmed, F.I., Yahaya, M., Adamu, A., Yaqub, Y., Dayyab, F.M., Edwin, C.E., Garba, S. and Gadzama, G.B. (2021). Carbapenem-resistant *Enterobacteriaceae* infections among patients admitted to intensive care units in Kano, Nigeria. SMJ 24:1-9.

Callejon, R. M., Rodriguez´ıguez-Naranjo, M. I., Ubeda, C., Hornedo-Ortega, R., Garcia-Parrilla, M. C. and Troncoso, A. M. 2015. "Reported foodborne outbreaks due to fresh produce in the United States and European Union: trends and causes," *FPD*, vol. 12(1):32–38.

Cheesebrough, M. (2002). District laboratory practice in tropical countries Part two, 2nd Edn. Cambridge University Press, U.KU.K. pp. 143–180.

Clinical and Laboratory Standards Institute. 2019. *M100-S29: Performance Standards for Antimicrobial Susceptibility Testing*. Wayne, PA: Clinical and Laboratory Standards Institute

Goodburn, C. & Wallace, C. A. (2013). "The microbiological efficacy of decontamination methodologies for fresh produce: a review," *Food Control*, 32(2):418–427.

Lamba, M., Graham, D. W. and Ahammad, S. Z. (2017). Hospital wastewater releases of carbapenem-resistance pathogens and genes in urban India. *EST* 51, 13906–13912. doi: 10.1021/acs.est.7b03380

Liu, B. T., Song, F. J., Zou, M., Zhang, Q. D. & Shan, H. (2017). High incidence of *Escherichia coli* strains coharboring *mcr-1* and *bla*NDM from chickens. *AAC,* 61:e02347-16. DOI: 10.1128/AAC.02347-16

Lodise, T., Ye, M. J. and Zhao, Q. (2017). Prevalence of invasive infections due to carbapenem-resistant *Enterobacteriaceae* among adult patients in U.S.U.S. Hospitals*. AAC,* 61:e00228-17. DOI: 10.1128/AAC.00228-17

Mir, S. A., Shah, M. A., Mir, M. M., Dar, B. N., Greiner, R. and Roohinejad, S. 2018. "Microbiological contamination of ready-toeat vegetable salads in developing countries and potential solutions in the supply chain to control microbial pathogens," *Food Control*, 85:235–244.

Morrison, B. J. and Rubin, J. E. (2015). Carbapenemase producing bacteria in the food supply escaping detection. *PLoS ONE* 10:e0126717. doi: 10.1371/journal.pone.0126717

Nair, P.K. and Vaz, M.S. (2013). Prevalence of carbapenem resistant Enterobacteriaceae from a tertiary care hospital in Mumbai, India Journal of Microbiology and Infectious Diseases 3 (4): 207-210 JMID doi: 10.5799/ahinjs.02.2013.04.0110

Ogbolu, D.O. and Webber, M.A. (2014). High-level and novel mechanisms of carbapenem resistance in Gram-negative bacteria from tertiary hospitals in Nigeria. *IJAA,* 43: 412–417.

Olalekan, A., Onwugamba, F., Iwalokun, B., Mellmann, A., Becker, K. and Schaumburg, F. (2020). High proportion of carbapenemaseproducing *Escherichia coli* and *Klebsiella pneumoniae* among extended-spectrum β-lactamaseproducers in Nigerian hospitals. *IJAR,* 21: 8–12.

Olowo-okere, Y. K. E., Ibrahim, B. O., Olayinka, J. O., Ehinmidu, Y., Mohammed, L., Nabti, Z., Rolain, J.-M. and Diene, S. M. (2020). Phenotypic and genotypic characterization of clinical carbapenem resistant Enterobacteriaceae isolates from Sokoto, northwest Nigeria. NMNI, 1;37:100727. doi: 10.1016/j.nmni.2020.100727. PMID: 32939286; PMCID: PMC7479348 37 (C).

Septembre-Malaterre, A., Remize, F. and Poucheret, P. 2018. "Fruits and vegetables, as a source of nutritional compounds and phytochemicals: changes in bioactive compounds during lactic fermentation," *FRI* 104: 86–99.

Sfeir, M.M., Hayden, J.A., Fauntleroy, K.A., Mazur, C., Johnson, J.K., Simner, P.J., Das, S., Satlin, M.J., Jenkins, S.G. and Westblade, L.F. (2019). EDTAmodified carbapenem inactivation method: a phenotypic method for detecting metallo-βlactamase-producing Enterobacteriaceae. JCM 57:e01757-18. https://doi.org/10 .1128/JCM.01757-18

Singh-Moodley, A. and Perovic, O. (2016). Antimicrobial susceptibility testing in predicting the presence of carbapenemase genes in

Enterobacteriaceae in South Africa. *BMC Infectious Diseases* 16:536. DOI: 10.1186/s12879-016- 1858-7

Tsai, Y., Wang, S., Chiu, H., Kao, C. and Li-Li. (2020). Combination of modified carbapenem inactivation method (mCIM) and EDTA-CIM (eCIM) for phenotypic detection of carbapenemase-producing Enterobacteriaceae. BMC Microbiology 20:315 https://doi.org/10.1186/s12866-020-02010-3.

van der Zwaluw, K., de Haan, A., Pluister, G.N., Bootsma, H.J., de Neeling, A.J. and Schouls, L.M. (2015) The Carbapenem Inactivation Method (CIM), a Simple and Low-Cost Alternative for the Carba NP Test to Assess Phenotypic Carbapenemase Activity in GramNegative Rods. PLoS ONE 10(3): e0123690. doi:10.1371/journal.pone.0123690

Wang, J., Yao, X., Luo, J., Lv, L., Zeng, Z., and Liu, J. H. (2018). Emergence of *Escherichia coli* co-producing NDM-1 and KPC-2 carbapenemases from a retail vegetable, China. *J. Antimicrob. Chemother.* 73, 252–254. DOI: 10.1093/jac/dkx335

Wang, Y., Zhang, R., Li, J., Wu, Z., Yin, W., Schwarz, S., Tyrell, J.M., Zheng, Y., Wang, S., Shen, Z., Liu, Z., Liu, J., Lei, L., Li, M, Zhang, Q., Wu, C., Zhang, Q., Wu, Y., Walsh, T.R. and Shen, J. (2017). Comprehensive resistome analysis reveals the prevalence of NDM and MCR-1 in Chinese poultry production. *Nature Microbiology* 2:16260. doi: 10.1038/nmicrobiol.2016.260

Yahia, E. M., Garc´ıa-Sol´ıs, P. and Celis, M. E. M. 2019.*Contribution of Fruits and Vegetables to Human Nutrition and Health Postharvest Physiology and Biochemistry of Fruits and Vegetables*, Elsevier, Amsterdam, Netherlands.

Zhang, R., Liu, L., Zhou, H., Chan, E. W., Li, J., Fang, Y., Li, D., Liao, K. and Chen, S. (2017). Nationwide surveillance of clinical carbapenem-resistant Enterobacteriaceae (CRE) strains in China. *EBioMedicine* 19, 98–106. doi: 10.1016/j.ebiom.2017.04.032