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Azithromycin and Colistin Resistance in Carbapenem-resistant *Escherichia coli* Isolates from Iraq

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ABSTRACT

Azithromycin is a proper antibiotic for eradication of infections caused by Gram-negative species. Our aim was determination of azithromycin resistance levels among carbapenem-resistant *E. coli* (CR-*E. coli*). Two hundred *E. coli* were identified. The minimum inhibitory and bactericidal concentrations (MIC and MBC, respectively) of imipenem and azithromycin were determined using agar dilution method. PCR was implemented to verify the existence of resistance genes. One-hundred *E. coli* isolates were carbapenem-resistant. Thirty-five CR-*E. coli* and five carbapenem-susceptible *E. coli* (CS-*E. coli*) isolates were resistant to azithromycin, respectively. The azithromycin MIC ranged from 16-64 µg/ml and its MBC ranged from 32-64 µ/ml, respectively. The carbapenem resistance genes included bla_{IMP} (32%) and bla_{OXA-48} (3%) genes. Furthermore, azithromycin resistance genes included *mph* (A) (12% in CR-*E. coli* and 3% in CS-*E. coli*) and *erm* (A) (4% in CR-*E. coli*) genes. Three CR-*E. coli* isolates had concomitantly the bla_{OXA-48}, bla_{IMP}, *erm* (A) and *mph* (A) genes. None of them were resistant to colistin. Azithromycin resistant *E. coli* was most probably developed from CR-*E. coli* than CS-*E. coli*. Spread of these strains in the era of Corona virus pandemic was a crisis to eradicate multidrug-resistant (MDR) strains.

Key words: Azithromycin resistance, carbapenem-resistant *Escherichia coli*, Iraq

INTRODUCTION

Escherichia coli causing diarrhea is an agent of death in particular among children under five years (Liu *et al.*, 2015; Zenebe *et al.*, 2022). Its treatment is mainly performed using ampicillin and cotrimoxazole in developing countries (Gomes *et al.*, 2019). However, antibiotic resistance particularly against carbapenems and other classes of antibiotics has led to the failure in their eradication. Considering this, the surveillance of mechanisms of resistance and its levels is a necessity which contributes to design proper therapeutic approaches (Benmessaoud *et al.*, 2016; Joffré and Iñiguez Rojas, 2020; Manjate *et al.*, 2022). Carbapenem resistance is caused via several mechanisms such as efflux pumps and various β–lactamase enzymes. Macrolides have been used for the eradication of various bacterial species. However, these are utilized less commonly to eliminate the *Enterobacteriaceae* members because of low rate of penetration into the membrane (Gomes *et al.*, 2017; Larson *et al.*, 2019; Taneja and Sharma, 2019). Nonetheless, azithromycin has shown higher efficiency compared to other members of macrolides. Therefore, azithromycin plays a better role in the treatment of diarrheal infections due to the Enterobacteriaceae family such as *E. coli*. The breakpoints of azithromycin resistance have not been fully determined (Lübbert, 2016; Cohen *et al.*, 2017; Gomes *et al.*, 2017). However, inhibition diameter of ≤ 12 mm and MIC ≥ 32 mg/l has been used for some of Enterobacteriaceae members. Several mechanisms of macrolide resistance have been reported such as efflux pumps and amino acid variations in the ribosomal proteins L4 (*rplD*) and L22 (*rplV*) of 23S rRNA (rrlH). Nonetheless, those mechanisms of resistance in Enterobacteriaceae mostly include mutations in methylase, phosphorylase and esterase genes (*erm*, *mph* and *ere*) genes, respectively and mobile gene elements such as *msr* (A), *mef* (A) or *mef* (B) which encode efflux pumps (Du *et al.*, 2018; Leung *et al.*, 2019). In this study, the azithromycin resistance levels among carbapenem-resistant *E. coli* isolates causing diarrhea in Iraq were investigated.

MATERIALS AND METHODS

Two hundred diarrhogenic *E. coli* isolates were identified using common biochemical and polymerase chain reaction (PCR, for amplification of *rep* and *uidA* genes) tests. The patients age ranged from 1-56 years (mean = 41.3±3); 114 of them being females and 86 cases being males.

The antibiotic resistance was performed using disk diffusion test as per clinical and laboratory standards institute (CLSI). The antibiotics included amoxicillin, gentamicin, azithromycin, cefepime, ceftazidime, colistin, imipenem, tetracycline and tigecycline (Wacker *et al.*, 2014; Palma *et al.*, 2017; Humphries *et al.*, 2021). The minimum inhibitory and bactericidal concentrations (MIC and MBC), respectively of imipenem and azithromycin were determined using agar dilution method. The range of antibiotic concentrations included from 0.5-128 µg/ml.

The PCR was performed to detect the resistance genes including bla_{IMP}, bla_{OXA-48}, *ere* (A), *erm* (A), *mef* (A), *mef* (B), *mph* (A), *mph* (B), *msr* (A) and *msr* (D) genes using primers listed in Table 1 as described by Palma *et al.* (2017). For strains carrying all the genes were used as positive controls. The sequence of primers used in this study was adopted from Palma *et al.* (2017).

Table 1. The sequence of primers used in this study[15]

Primer	Sequence 5' to 3'	Annealing T (°C)	Product size
bla_{IMP1}	F: GGGTGGGGCGTTGTTCCTA R: TCTATTCCGCCCGTGCTGTC	62	198
bla_{OXA-48}	F: CGCCCGCGTCGACGTTCAAGAT R: TCGGCCAGCAGCGGATAGGACAC	65	484
erm (A)	F: TCTAAAAAGCATGTAAAAGAAA R: CGATACTTTTTGTAGTCCTTC	52	533
erm (B)	F: GAAAAAGTACTCAACCAAATA R: AGTAACGGTACTTAAATT	45	639
erm (C)	F: TCAAAACATAATATAGATAAA R: GCTAATATTGTTTAAATCGTCAAT	45	642
mph (A)	F: GTGAGGAGGAGCTTCGCGAG R: TGCCGCAGGACTCGGAGGTC	60	403
mph (B)	F: ATTAAACAAGTAATCGAGATAGC R: TTTGCCATCTGCTCATATTCC	868	50
msr (A)	F: GCACTTATTGGGGGTAATGG R: GTCTATAAGTGCTCTATCGTG	384	58
ere (A)	F: GCCGGTGCTCATGAACTTGAG R: CGACTCTATTCGATCAGAGGC	420	60
mef (A)	F: AGTATCATTAATCACTAGTGC R: TTCTTCTGGTACTAAAAGTGG	345	54
mef (B)	F: ATGAACAGAATAAAAAATTG R: AAATTATCATCAACCCGGTC	1255	45

RESULTS AND DISCUSSION

The patients' age ranged from 1-78 years with mean of 56.5±4.3. The males and females rate included 120 and 80, respectively. Risk factors such as prior antibiotic use (n=140; 70%) and hospitalization (n=160, 80%) were detected to be significant. All the isolates were resistant to amoxicillin and ceftazidime, followed by tetracycline (96%), cefepime (82%), imipenem (50%), gentamicin (45%), azithromycin (20%) and tigecycline (2%). None of them was resistant to colistin. Therefore, 45% of isolates were multidrug-resistant (MDR) *E. coli*. Out of 100 CR-*E. coli* isolates, the imipenem MIC and MBC ranges included 16-128 and 32-128 µg/ml, respectively. Thirty-five CR-*E. coli* and five carbapenem-susceptible *E. coli* (CS-*E. coli*) isolates were resistant to azithromycin, respectively. The azithromycin MIC ranged from 16-64 µg/ml and its MBC ranged from 32-64 µ/ml, respectively.

The carbapenem resistance genes included bla_{IMP} (32%) and bla_{OXA-48} (3%) genes. Furthermore, azithromycin resistance genes included *mph* (A) (12% in CR-*E. coli* and 3% in CS-*E. coli*) and *erm* (A) (4% in CR-*E. coli*) genes (Fig. 1 to 4). Three isolates had concomitantly the bla_{OXA-48}, bla_{IMP}, *erm* (A) and *mph* (A) resistance genes (Table 2).

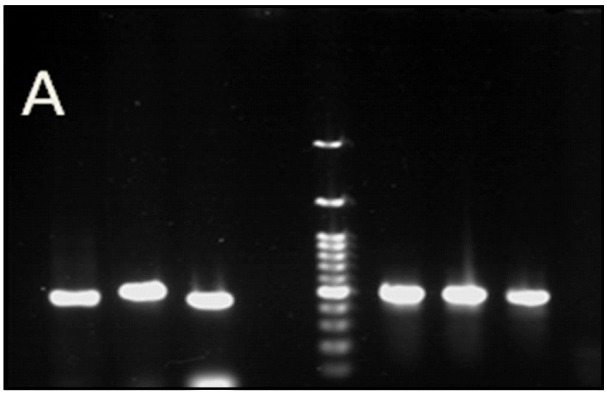

Fig. 1. The products of resistance genes amplification; A: the bla_{OXA-48} with 484bp.

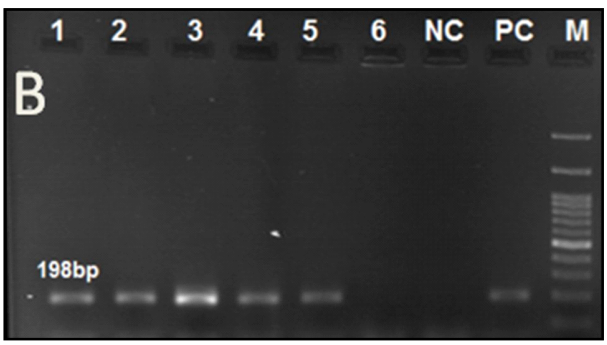

Fig. 2. The products of resistance genes amplification; B: The bla_{IMP} with 198bp.

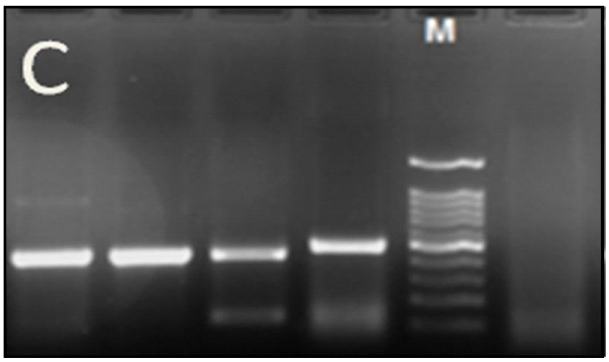

Fig. 3. The products of resistance genes amplification; C: The erm (A) with 533bp.

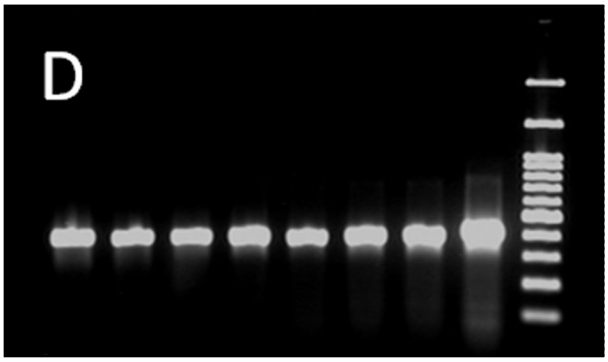

Fig. 4. The products of resistance genes amplification; D: the mph (A) with 403bp

Where: Amx: amoxicillin, Te: tetracycline, FEP: cefepime, CAZ: ceftazidime, IMP: imipenem, Az: azithromycin and TG: tigecycline.

Deaths due to pathogenic *E. coli* is among most challenges worldwide, particularly in developing countries (Alkhudhairy *et al.*, 2019). Development of resistance to commonly used and last-line antibiotics is a concern causing failure in therapies, hence, alternative choices seem necessitate. In this study, it was observed that lower than half of *E. coli* were resistant to azithromycin, while CR-*E. coli* was significantly more common to be resistant than CS-*E. coli*. Notably, there were significant risk factors including prior antibiotic consumption and hospital residence among patients. These risk factors have been reported as significant determinants in the acquisition or spread of MDR isolates (Ghasemian *et al.*, 2018; Alkhudhairy *et al.*, 2019). Out of 100 CR-*E. coli* isolates, the imipenem MIC and MBC included 16-128 and 32-128 µg/ml, respectively. Thirty-five CR-*E. coli* and five carbapenem-susceptible *E. coli* (CS-*E. coli*) isolates were resistant to azithromycin, respectively. The azithromycin MIC ranged from 16-64 µg/ml and its MBC ranged from 32-64 µg/ml, respectively. The carbapenem resistance genes included bla_{IMP} (32%) and bla_{OXA-48} (3%) genes. During recent years, the CR-*E. coli* carrying various carbapenemase genes have been reported from most of areas worldwide. It was observed that 20% of isolates were azithromycin resistant and related genes included *mph* (A) (12% in CR-*E. coli* and 3% in CS-*E. coli*) and *erm* (A) (4% in CR-*E. coli*) genes. The azithromycin MIC among those isolates carrying the *ere* (A) gene ranged from 16-64 µg/ml. There was significant higher azithromycin resistance rate among CR-*E. coli* than CS-*E. coli*. Furthermore, of 40 patients infected with azithromycin-resistant *E. coli*, 87.5% (n=35, $p<0.0001$) of them had previous carbapenem consumption. Efflux pumps play a crucial role in azithromycin resistance (Gomes *et al.*, 2019; Manoharan-Basil *et al.*, 2021; Pushpker *et al.*, 2022). Moreover, DNA mutations may also have a role in this regard (Manoharan-Basil *et al.*, 2021). It has been reported that *mph* (A) gene plays a crucial role in the azithromycin resistance. Moreover, the *msr* (D) gene has been associated with the resistance, nonetheless, it was not detected in this study (Lluque *et al.*, 2015; Ma *et al.*, 2017; Washington *et al.*, 2021). Reports regarding the role of *erm* (A) gene are scarce in this region, while for the first time it was detected in eight CR-*E. coli* with MIC range of 16-64 µg/ml (Marosevic *et al.*, 2017; Washington *et al.*, 2021). It has been stated that the *ere* (A) gene has a minimal role in the azithromycin resistance and it was not

Table 2. The characteristics and risk factors associated with the existence of *E. coli* isolates carrying the bla_{OXA-48}, bla_{IMP}, *erm* (A) and *mph* (A) genes

Isolate	Patient age/ gender	Prior antibiotic use	Resistance	MIC_{IMP}	MIC_{AZ}
1	72/female	Yes	Amx, Te, FEP, CAZ, IMP, Az, TG	64	32
2	64/male	Yes	Amx, Te, FEP, CAZ, IMP, Az, TG	64	64
3	71/female	Yes	Amx, Te, FEP, CAZ, IMP, Az	32	64

Where: Amx: amoxicillin, Te: tetracycline, FEP: cefepime, CAZ: ceftazidime, IMP: imipenem, Az: azithromycin and TG: tigecycline.

detected in this study (Gomes *et al.*, 2019; Zielinski *et al.*, 2021). Notably, most of studies have concluded that the *mph* (B) is unable to hydrolyse the azithromycin and erythromycin (Golkar *et al.*, 2018). Previously, it has been reported that commensal *E. coli* plays an important role as reservoir of macrolide resistance genes (MRGs) (Gomes *et al.*, 2019). The MIC of azithromycin among those isolates which concomitantly carried the *mph* (A) and *erm* (A) included 32-64 µg/ml which highlight the central role of *mph* (A) gene in high level resistance.

CONCLUSION

It was observed that azithromycin resistant-*E. coli* was probably developed from CR-*E. coli* that CS-*E. coli*. Spread of these strains in the era of Corona virus pandemic was a crisis to eradicate MDR strains. Transferable resistance genes were associated with high azithromycin MIC rates. Combination therapies were suggested to hinder the development and spread of this kind of resistance.

REFERENCES

Alkhudhairy, M. K., Alshadeedi, S. M., Mahmood, S. S., Al-Bustan, S. A. and Ghasemian, A. (2019). Comparison of adhesin genes expression among *Klebsiella oxytoca* ESBL-non-producers in planktonic and biofilm mode of growth and imipenem sublethal exposure. *Microbial Patho.* **134**: 103558. *https://doi.org/10.1016/j.micpath.2019.103558.*

Benmessaoud, R., Nezha, M., Moraleda, C., Jroundi, I., Tligui, H., Seffar, M., Pons, M. J., Alvarez, M. J., Chaacho, S., Vila, J., Alonso, P. L., Bassat, Q. and Ruiz, J. (2016). Antimicrobial resistance levels among diarrhoeagenic micro-organisms recovered from children under-5 with acute moderate-to-severe diarrhoea in Rabat, Morocco. *J. Global Antimicrobial Res.* **7**: 34-36. *https://doi.org/10.1016/j.jgar.2016.07.005.*

Cohen, R., Raymond, J. and Gendrel, D. (2017). Antimicrobial treatment of diarrhea/acute gastroenteritis in children. *Archives de Pédiatrie* **24**: S26-S29. *https://doi.org/10.1016/S0929-693X(17)30515-8.*

Du, D., Wang-Kan, X., Neuberger, A., Van Veen, H. W., Pos, K. M., Piddock, L. J. and Luisi, B. F. (2018). Multidrug efflux pumps: Structure, function and regulation. *Nature Rev. Microbiol.* **16**: 523-539. *https://doi.org/10.1038/s41579-018-0048-6.*

Ghasemian, A., Mobarez, A. M., Peerayeh, S. N., Abadi, A. T. B., Khodaparast, S. and Nojoomi, F. (2018). Report of plasmid-mediated colistin resistance in Klebsiella oxytoca from Iran. *Rev. Med. Microbiol.* **29**: 59-63. *https://doi.org/10.1097/MRM.0000000000000134.*

Ghasemian, A., Rizi, K. S., Vardanjani, H. R. and Nojoomi, F. (2018). Prevalence of clinically isolated metallo-beta-lactamase-producing *Pseudomonas aeruginosa*, coding genes and possible risk factors in Iran. *Iranian J. Path.* **13**, 1.*https://doi.org/10.30699/ijp.13.1.1.*

Golkar, T., Zielinski, M. and Berghuis, A. M. (2018). Look and outlook on enzyme-mediated macrolide resistance. *Front. Microbiol.* **9**: 1942. *https://doi.org/10.3389/fmicb.2018.01942.*

Gomes, C., Martínez-Puchol, S., Palma, N., Horna, G., Ruiz-Roldán, L., Pons, M. J. and Ruiz, J. (2017). Macrolide resistance mechanisms in Enterobacteriaceae: Focus on azithromycin. *Crit. Rev. Microbio.* **43**: 01-30. *doi.org/10.3109/1040841X.2015.1136261.*

Gomes, C., Ruiz-Roldán, L., Mateu, J., Ochoa, T. J. and Ruiz, J. (2019). Azithromycin resistance levels and mechanisms in *Escherichia coli*. *Scient. Rep.* **9**: 6089. *https://doi.org/10.1038/s41598-019-42423-3.*

Humphries, R., Bobenchik, A. M., Hindler, J. A. and Schuetz, A. N. (2021). Overview of changes to the clinical and laboratory standards institute performance standards for antimicrobial susceptibility testing, M100. *J. Clin. Microbiol.* **59**: e00213-21. *https://doi.org/10.1128/JCM.00213-21.*

Joffré, E. and Iñiguez Rojas, V. (2020). Molecular epidemiology of enteroaggregative Escherichia coli (EAEC) isolates of hospitalized children from Bolivia reveal high heterogeneity and multidrug-resistance. *Int. J. Mol. Sci.* **21**: 9543. *https://doi.org/10.3390/ijms21249543.*

Larson, A., Hartinger, S. M., Riveros, M., Salmon-Mulanovich, G., Hattendorf, J., Verastegui, H., Huaylinos, M. L. and Mäusezahl, D. (2019). Antibiotic-resistant Escherichia coli in drinking water samples from rural Andean households in Cajamarca, Peru. *Am. J. Trop. Med. Hygiene* **100**: 1363. *https://doi.org/10.4269/ajtmh.18-0776.*

Leung, A. K., Leung, A. A., Wong, A. H. and Hon, K. L. (2019). Travellers' diarrhea: A clinical review. *Recent Patents Infl. Allergy Drug Disc.* **13**: 38-48. *https://doi.org/10.2174/1872213X13666190514105054.*

Liu, L., Oza, S., Hogan, D., Perin, J., Rudan, I., Lawn, J. E., Cousens, S., Mathers, C. and

Black, R. E. (2015). Global, regional and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: An updated systematic analysis. *The Lancet* **385**: 430-440. *https://doi.org/10.1016/S0140-6736(14)61698-6.*

Lluque, A., Mosquito, S., Gomes, C., Riveros, M., Durand, D., Tilley, D. H., Bernal, M., Prada, A., Ochoa, T. J. and Ruiz, J. (2015). Virulence factors and mechanisms of antimicrobial resistance in Shigella strains from periurban areas of Lima (Peru). *Int. J. Med. Microbiol.* **305**: 480-490. *https://doi.org/10.1016/j.ijmm.2015.04.005.*

Lübbert, C. (2016). Antimicrobial therapy of acute diarrhoea: A clinical review. *Expert Rev. Anti-Infective Ther.* **14**: 193-206. *https://doi.org/10.1586/14787210.2016.1128824.*

Ma, Q., Xu, X., Luo, M., Wang, J., Yang, C., Hu, X., Liang, B., Wu, F., Yang, X., Wang, J., Liu, H., Li, W., Zhong, Y., Li, P., Xie, J., Jia, L., Wang, L., Hao, R., Du, X., Qiu, S., Song, H. and Sun, Y. (2017). A waterborne outbreak of Shigella sonnei with resistance to azithromycin and third-generation cephalosporins in China in 2015. *Antimicrobial Agen. Chemo.* **61**: e00308-17. *https://doi.org/10.1128/AAC.00308-17.*

Manjate, F., Quintó, L., Chirinda, P., Acácio, S., Garrine, M., Vubil, D., Nhampossa, T., João, E. D., Nhacolo, A., Cossa, A., Massora, S., Bambo, G., Bassat, Q., Kotloff, K., Levine, M., Alonso, P. L., Tate, J. E., Parashar, U., Mwenda, J. M. and Mandomando, I. (2022). Impact of rotavirus vaccination on diarrheal hospitalizations in children younger than 5 years of age in a rural southern Mozambique. *Vaccine* **40**: 6422-6430. *https://doi.org/10.1016/j.vaccine.2022.09.050.*

Manoharan-Basil, S. S., Laumen, J. G. E., Van Dijck, C., De Block, T., De Baetselier, I. and Kenyon, C. (2021). Evidence of horizontal gene transfer of 50S ribosomal genes rplB, rplD, and rplY in Neisseria gonorrhoeae. *Front. Microbiol.* **12**: 683901. *https://doi.org/10.3389/fmicb.2021.683901.*

Marosevic, D., Kaevska, M. and Jaglic, Z. (2017). Resistance to the tetracyclines and macrolidelincosamide-streptogramin group of antibiotics and its genetic linkage–A review. *Ann. Agric. Environ. Med.* **24**. *https://doi.org/10.26444/aaem/74718.*

Palma, N., Pons, M. J., Gomes, C., Mateu, J., Riveros, M., García, W., Jacobs, J., García, C., Ochoa, T. J. and Ruiz, J. (2017). Resistance to quinolones, cephalosporins and macrolides in Escherichia coli causing bacteraemia in Peruvian children. *J. Global Antimicrob. Res.* **11**: 28-33. *https://doi.org/10.1016/j.jgar.2017.06.011.*

Pushpker, R., Bay, D. C. and Turner, R. J. (2022). Small multidrug resistance protein EmrE phenotypically associates with OmpW, DcrB and YggM for osmotic stress protection by betaine in *Escherichia coli*. *Microbiol.* **168**, 001287. *https://doi.org/10.1099/mic.0.001287.*

Taneja, N. and Sharma, M. (2019). Antimicrobial resistance in the environment: The Indian scenario. *Ind. J. Med. Res.* **149**: 119-128. *DOI: 10.4103/ijmr.IJMR_331_18.*

Wacker, M., Wang, L., Kowarik, M., Dowd, M., Lipowsky, G., Faridmoayer, A., Shields, K., Park, S., Alaimo, C., Kelley, K. A., Braun, M., Quebatte, J., Gambillara, V., Carranza, P., Steffen, M. and Lee, J. C. (2014). Prevention of *Staphylococcus aureus* infections by glycoprotein vaccines synthesized in *Escherichia coli*. *The J. Inf. Dis.* **209**: 1551-1561. *https://doi.org/10.1093/infdis/jit800.*

Washington, M. A., Taitt, C. R., Blythe, J., Hering, K. and Barnhill, J. (2021). *Escherichia coli* as a potential reservoir of antimicrobial resistance genes on the Island of O 'ahu. *Hawai'i J. Health Soc. Welf.* **80**: 9. *https://doi.org/10.3390/antibiotics10040351.*

Zenebe, T., Eguale, T., Mihret, A. and Abebe, T. (2022). Relative burden of diarrheal cases in under-five children before and during COVID-19 pandemic in Ethiopia: A retrospective study. *Res. Squ.* **9**: 1-20. *https://doi.org/10.21203/rs.3.rs-1756422/v1.*

Zielinski, M., Park, J., Sleno, B. and Berghuis, A. M. (2021). Structural and functional insights into esterase-mediated macrolide resistance. *Nat. Comm.* **12**: 1732. *https://doi.org/10.1038/s41467-021-22016-3.*