

ANTIBIOGRAM AND DETECTION OF METALLO- β -LACTAMASE PRODUCING *Escherichia coli* ISOLATED FROM COW DUNG IN OWO METROPOLIS

ABSTRACT

This study was carried out to determine the antibiotic susceptibility profile and the production of metallo beta lactamase (MBL) by *Escherichia coli* isolated from cow dung in Owo metropolis. The isolation of *Escherichia coli* was done using MacConkey agar and Eosin Methylene Blue Agar and were conventionally characterized. Antimicrobial susceptibility test of the isolates were by disc diffusion method against ceftazidime (30 μ g), cefuroxime (30 μ g), gentamicin (10 μ g), cefixime (5 μ g), ofloxacin (5 μ g), augmentin (30 μ g), nitrofurantoin (300 μ g) and ciprofloxacin (5 μ g). Detection of MBL producing isolates was by imipenem-EDTA combined disc test. The isolates showed highest resistance to augmentin (97.8%) and least resistance to nitrofurantoin (20.0%). Out of the 45 *Escherichia coli* isolated from cow dung, 8 (17.8%) produced MBL and they were all multidrug resistant. The production of MBL and the high prevalence of antibiotic resistance observed among the *Escherichia coli* in this study infer that cow dung does not only serves as a reservoir for MBL-producers but also as source for the growth and dissemination of clinically significant antibiotics resistance among bacteria species. Hence, the use of antibiotics as growth enhancers in cow production should be discouraged to help prevent the spread of antibiotic resistant bacteria and thus, preserve the efficacy of available antibiotics.

Keywords: Metallo- β -lactamases; *Escherichia coli*; Antibiotics resistance; Owo

INTRODUCTION

Emergence and rapid dissemination of antibiotic resistance in bacteria is now a global concern. The antibiotic resistance acquired by organisms in one ecosystem can easily be transferred among organisms in different ecosystems (Levy, 1997; Hasan *et al.*, 2012). This, in turn, is responsible for wide-scale epidemic and endemic spreads of multidrug-resistant bacteria. Now, it is evident that resistant microbes are found in the different environmental compartments and reservoirs due to misuse and overuse of antibiotics and poor health-care infrastructures (Bonnedahl *et al.*, 2010; Martinez, 2008). Surprisingly, antibiotic-resistant bacteria were found in the pristine environments where there was no direct human influence like habitation, farming, and hospitals (Hernandez *et al.*, 2012; Sjolund *et al.*, 2008). Antibiotic-resistant Bacteria were even found in the soil when raw cow dung and manure were used extensively as fertilizers (Sahoo *et al.*, 2012) and ultimately spread into natural and drinking water sources (da Costa *et al.*, 2013).

E. coli has been noted as a very important foodborne pathogen and as the etiological agent of different extra intestinal infections, like urinary tract infection and septicemia (Zhong *et al.*, 2019; Bonten *et al.*, 2021). Salyers *et al.* (2004) opined that *E. coli* of faecal origin may function as a reservoir of genes coding for drug resistance, and are usually regarded a potential indicator of acquired antibiotic resistant genes in an environment (Nys *et al.*, 2004). There are reports of bacterial resistance to commercially available and frequently administered antibiotics in the developing nations around the world. Moreover, variation exists in the pattern of resistance exhibited by *E. coli* isolated from the faecal samples of apparently healthy children (Mahmoodi *et al.*, 2020; Ferjani *et al.*, 2018; Shakya *et al.*, 2013; Dyar *et al.*, 2012; Sahoo *et al.*, 2012; Bartoloni *et al.*, 2006).

Carbapenems generally are presently used as last line antibiotics for the management of infections caused by Gram negative bacteria particularly those suspected to be multidrug-resistant (Oli *et al.*, 2019; Ali *et al.*, 2020; Hosuru *et al.*, 2020). Before now, members of the Family Enterobacteriaceae were not resistant to carbapenems, albeit this has changed because of the appearance of some carbapenem resistant enteric bacteria in the last decade thereby making them a serious public health issue (Hosuru *et al.*, 2020). The 2013 report of the United States Center for Disease Control and Prevention (CDC) shows that the emergence of enteric bacteria resistant to carbapenem, in the past few years remains the main cause of hard-to-manage infections in patients admitted in hospitals, and they are referred to as considered a pressing danger to human health (Oli *et al.*, 2019; Hosuru *et al.*, 2020).

Metallo beta lactamase enzymes also known as carbapenemases which hydrolyzes carbapenems, and possess powerful action on other antibiotics in the class of beta-lactam apart from monobactams (Iyobe *et al.*, 2001). They are also recognized to bestow different level of resistance to beta-lactam antibiotics and their availability in Gram negative organisms particularly those of clinical importance has subjected the usage of carbapenems under risk (Tortola *et al.*, 2005 and Thompson, 2010). Metallo Beta-Lactamase (MBLs)-producing *E. coli* are of paramount concern because they are multidrug resistant, limit therapeutic options, lead to treatment failures and increase the cost for treatment and duration of hospitalization.

Currently, there is paucity of information on MBL-producing *E. coli* from animals and the possible contribution of these resistant species to the ever growing antimicrobial resistance

observed in humans. Hence, this study was carried out to determine the antibiogram and the production of MBL by *E. coli* isolated from cattle dung in Owo metropolis.

Materials and Methods

Sample Collection: Between January and February 2022, freshly passed cattle dung were aseptically collected at the Central Abattoir, Owo in Ondo State from apparently healthy cattle which were about to be slaughtered into appropriately labelled sterile capped universal bottles with sterile spatula, preserved in ice packs and transported to Microbiology unit of the Department of Science Laboratory Technology, Rufus Giwa Polytechnic Owo for immediate analyses.

Isolation of *Escherichia coli*: one gram of the cow dung sample was weighed into 10 ml of de-ionized water to make a stock solution. From the stock tenfold serial dilution was carried out. 1ml each of the serial diluents (10^{-2} , 10^{-4} and 10^{-6}) was dispensed into appropriately labeled sterile Petri dishes. Aseptically, MAC and EMB Agar cooled to about 45 °C was separately dispensed into the aliquots of samples in the Petri dishes and swirled gently, allowed to solidify and incubated at 35-37 °C for 18-24 hours (Egea *et al.*, 2012). Distinct colonies were sub-cultured on newly prepared nutrient agar dishes; repeated streaking was done to obtain pure culture of *Escherichia coli* prior to biochemical tests (Cheesbrough, 2010).

Preparation and Standardizing Inoculum Suspension: The inoculum suspension was prepared by picking 2-3 colonies of a 24-hour culture with a sterile wire loop and was suspended in 5M normal saline, the suspension was mixed with a vortex mixer. The turbidity of the suspension was standardized to match the 0.5 McFarland's standard which corresponds to approximately 1.5×10^8 cfu/ml and this was done by comparing the test suspension with barium sulphate suspension by placing the tubes in front of a white paper with black lines.

Antimicrobial susceptibility test of the isolated *Escherichia coli*: Antibiotic susceptibility test on the *Escherichia coli* was done using disc-agar diffusion technique (CLSI, 2018). The test antibiotics employed were the following classes of antibiotic agent β -lactam combinations (amoxicillin/clavulanate 20/10 μ g), cephem (cefotaxime 30 μ g, ceftazidime 30 μ g, cefixime 5 μ g), carbapenem (imipenem 10 μ g), aminoglycosides (gentamicin 10 μ g), fluoroquinolone (ciprofloxacin 5 μ g, ofloxacin 5 μ g), and nitrofurans (nitrofurantoin 300 μ g). After incubation period, zones of inhibition were measured in millimeter while the protocols in CSLI (2018)

guidelines were used for the interpretation. The zones of inhibition obtained were compared with reference for proper classification of the organisms as sensitive, intermediate or resistant to specific antibiotics (CLSI, 2018). Any isolate that shows resistance to minimum of two different classes of antibiotics was taken as a multidrug resistant strain (Magiorakos *et al.*, 2012).

Isolates' MBL production screening

All isolates that were resistant to imipenem by recording a zone of inhibition diameter less than 23 mm were suspected of producing the enzyme metallo- β -lactamase (Aibinu *et al.*, 2007). They were further subjected to confirmation test phenotypically.

Phenotypic confirmation of MBL-positive isolates

The turbidity of a culture of the isolated *E. coli* was adjusted to 0.5 MacFarland standard, then inoculated aseptically on freshly prepared Muller-Hinton agar dishes. Later, antibiotic disk containing 10 μ g imipenem and infused with 1 μ g of EDTA as well as another imipenem disc free of EDTA were placed aseptically on the freshly inoculated agars. The dishes were incubated for 24 h at 30 °C, then inhibition zones were taken and interpreted using the criteria set by CLSI (2018). According to Ejikeugwu *et al.* (2016), a variation of 7 mm or more recorded in the zones of inhibition between the imipenem disc infused with EDTA and disc without EDTA confirms the phenotypic production of MBL.

Results and Discussion

Results

Identification of the isolates

Table 1 showed that all the isolates were Gram-negative rods. They were motile, catalase, indole, methyl red, glucose and lactose positive but were citrate, Voges-Proskauer and maltose negative.

Antibiotic susceptibility patterns of the isolated *E. coli* from cow dung

Table 2 showed the varying levels of susceptibility and resistance exhibited by the *E. coli* isolated from cow dung to the test antibiotics. The *E. coli* isolates from the cow dung showed highest resistance to augmentin (97.8 %) and least resistance of 20.0 % to nitrofurantoin.

Distribution of MBL-producing *E. coli* isolated from cow dung

Table 3 showed that from the 9 cow dung samples, 45 *Escherichia coli* isolates were obtained. Out of these, 13 (28.9 %) were suspected to be MBL-producers, 8 (17.8 %) were confirmed to be actual producers of MBL while 37 (82.2 %) were non-producers of MBL.

Antibiotype of the MBL-producing *E. coli*

The Antibiotypes of the MBL-producing *E. coli* showed that three (37.5 %) of the eight isolates that produced MBL resisted six (NIT-CIP-IMP-CAZ-GEN-AUG), another three (37.5 %) resisted five (CIP-IMP-GEN-CRX-AUG) and two (25 %) four (CIP-CAZ-GEN-AUG) different classes of antibiotics respectively, hence they were multidrug resistance.

Discussion

Different bacteria strains uses various mechanisms to resist the action of antibiotics, one of these mechanisms is the production of MBL particularly some species in the family *Enterobacteriaceae*. This kind of resistance mechanism has a great implication on public health because they limit available treatment alternatives for infections caused by bacteria that are multidrug resistant (Bashir *et al.*, 2011).

Table 1: Gram reaction and biochemical characterization of the isolates from the cow dung

Isolates	Shape	Gram Reaction	MOT	CAT	CIT	IND	MR	VP	GLU	LAC	MAL	Morphological Appearance on Culture Media	Probable Organism
1-45	Rod	-	+	+	-	+	+	-	+	+	-	Lactose-fermenting (pinkish) colonies on MAC; and green metallic sheen colonies on EMB	<i>Escherichia coli</i>

KEY: + = Positive, - = Negative, MOT = Motility, CAT = Catalase, OXI = Oxidase, CIT = Citrate, IND = Indole, MR = Methyl red, VP = Voges-proskauer, GLU = Glucose, LAC = Lactose, MAL = Maltose, MAC = MacConkey Agar, EMB = Eosin Methylene Blue Agar

Table 2: Antibiotic susceptibility patterns of all the *E. coli* isolated from cow dung

S/N	Antibiotics (µg)	No. (%) susceptibility	No. (%) resistance
1	AUG (30)	1 (2.2)	44 (97.8)
2	OFL (5)	13 (28.9)	32 (71.1)
3	CAZ (30)	2 (4.4)	43 (95.6)
4	CRX (30)	4 (8.9)	41 (91.1)
5	GEN (10)	15 (33.3)	30 (66.7)
6	CXM (5)	6 (13.3)	39 (86.7)
7	NIT (300)	36 (80.0)	9 (20.0)
8	CPR (5)	9 (20.0)	36 (80.0)
9	IMP (10)	32 (71.1)	13 (28.9)

KEY: S/N = Serial number, No. = Number, AUG = Augmentin, OFL = Ofloxacin, CAZ = Ceftazidime, CRX = Cefuroxime, GEN = Gentamisin, CXM = Cefixime, NIT = Nitrofurantoin, CPR = Ciprofloxacin, IMP = Imipenem

Table 3: Distribution of MBL-producing *E. coli* isolated from cow dung

Bacteria	Sample	No. of samples	No. of Isolates screened	No of suspected MBL isolates N (%)	MBL positive N (%)	MBL negative N (%)
<i>E. coli</i>	Cow dung	9	45	13 (28.9)	8 (17.8)	37 (82.2)

KEY: N = number of isolates, % = percentage, MBL = Metallo-Beta-Lactamase

Table 4: Antibiotype of MBL-producing *E. coli* isolated from cow dung

Antibiotype	Classes of Antibiotics	No. (%) MBL+ve <i>E. coli</i>
NIT-CIP-IMP-CAZ-GEN-AUG	6	3 (37.5)
CIP-IMP-GEN-CRX-AUG	5	3 (37.5)
CIP-CAZ-GEN-OFL-AUG	5	0 (0.0)
NIT-CIP-CXM-GEN-AUG	5	0 (0.0)
CIP-CRX-GEN-OFL-AUG	5	0 (0.0)
IMP-CXM-GEN-OFL	4	0 (0.0)
NIT-CRX-IMP-AUG	4	0 (0.0)
CIP-CAZ-GEN-AUG	4	2 (25)
CIP-CXM-OFX-AUG	4	0 (0.0)
CIP-CAZ-GEN-AUG	4	0 (0.0)
CAZ-OFX-AUG	3	0 (0.0)
CXM-GEN-AUG	3	0 (0.0)
CAZ-AUG	2	0 (0.0)
CPR-AUG	2	0 (0.0)
TOTAL		8

The result of the Gram reaction and characterization of the isolates in this study is in accordance with the characteristics of *Escherichia coli* as previously reported by other workers (Islam *et al.*, 2014). The result of the bacteriological investigation showed that a total of 45 *E. coli* isolates were gotten from the cow dung samples. This result varies a little with the report of Ejikeugwu *et al.* (2016) who isolated 32 *E. coli* from 40 cloacal swab samples of cow from a local abattoir in Abakaliki metropolis. This disparity may be as a result of difference in sampling techniques. Faecal-oral transmission is the major route through which pathogenic strains of the bacterium cause disease; and most *E. coli* strains are occasionally responsible for product recalls due to food contamination (Russell and Jarvis, 2001).

All the isolates gotten in this study revealed different degrees of resistance to the test commercially available antibiotics. Out of all the test antibiotics, the isolated *E. coli* showed special resistance to augmentine (97.8%), Ceftazidime (95.6%), Cefuroxime (91.1%), cefixime (86.7%), Ciprofloxacin (80.0%), Ofloxacin (71.1%) and gentamicin (66.7%). However, 71.1% of the isolates showed susceptibility to imipenem, an example of carbapenems. The reasonably high level of activity of the imipenem against the *E. coli* isolates in this study may help to

explain why carbapenems have remained the standard drug for the management infections occasioned by some Gram negative bacteria including those provoked by extended spectrum β -lactamases (Dahiya *et al.*, 2015; Thompson, 2010). The *E. coli* showed highest susceptibility to nitrofurantoin (80.0%), a nitrofuran. Similar findings have been reported by other researchers about the pattern of antibiotic resistance of *E. coli* isolated from different environments (Moore *et al.*, 2014; Ejikeugwu *et al.*, 2014; Ejikeugwu *et al.*, 2016).

The production of MBL was identified in 8 (17.8%) of the isolated *E. coli* from the total 45 isolates obtained in this research. There have been reports of the incidence of MBL producing *E. coli* in various settings; and these organisms are responsible for the wide spread of antibiotics resistance genes (Walsh *et al.*, 2005; Johnson *et al.*, 2013; Ejikeugwu *et al.*, 2014; Ejikeugwu *et al.*, 2016). In Owo local government area of Ondo State, Nigeria, the location of this present study, information on MBL positive *E. coli* isolated from animals is scarce. The challenge of widespread indiscriminate use and deposition of antibiotics in the environment may have been responsible for the spread of the resistance among many bacteria species. Moreover, other uses of antibiotics in animal production, fishery and other aquaculture as feed additives as well as their use for treating some diseases of plant may have contributed to the wide spread of antibiotic resistance among various microorganisms in our ecosystem (Claude, 2013).

The observation from this study that showed the MBL-producing *E. coli* exhibiting multiple drug resistance to a combination of six (NIT-CIP-IMP-CAZ-GEN-AUG), five (CIP-IMP-GEN-CRX-AUG) and four (CIP-CAZ-GEN-AUG) different classes of antibiotics respectively is similar and comparable to the report of a study carried out by Egbule and Yusuf (2019) on *E. coli* isolated from cattle and poultry faeces in Abraka, South-South Nigeria which shows multidrug resistance among the isolates, which may have been due to their expression of some enzymes that inactivate these drugs.

Conclusion

In conclusion, this present study has shown that the *E. coli* strains isolated from cow dung showed varying levels of susceptibility and resistance to different classes of antibiotics tested. The report of this study showed that some of the isolated *E. coli* produced metallo β -lactamase and this may have allowed them to resist the action of carbapenems. Hence, there is a need to create awareness about the danger inherent in indiscriminate use of antibiotics among the local people and particularly among the cattle rearers. Strict antibiotic policy and alternative measures

for rearing and production of food-producing animals (that does not include the use of antibiotics) is required now than ever to protect and sustain the efficacy of available antibiotics.

REFERENCES

- Aibinu, I., Nwanneka, T. and Odugbemi, T. (2007) Occurrence of ESBL and MBL in clinical isolates of *Pseudomonas aeruginosa* from Lagos Nigeria. *Journal of American Science*, 3, 81-85.
- Ali, M., Amal, J., Mohammed, A., Wael, M., Merin, G., Sadananda, A., Sanjay, R., Darshan, D.D., Chitra, J., Sajith, V., Aftab, A.K., Jilani, S. and Poojdev, J. (2020) Antimicrobial Resistance, Mechanisms and Its Clinical Significance. *Disease -A-Month*, 66, Article ID: 100971
- Bartoloni, A., Pallecchi, L., Benedetti, M., Fernandez, C., Vallejos, Y., Guzman, E., *et al.* (2006). Multidrug-Resistant Commensal *Escherichia coli* in Children, Peru and Bolivia. *Emerging Infectious Diseases*, 12 (6), 907–913.
- Bashir, D., Thokar, M. A., Fomda, B.A., Bashir, G., Zahoor, D., Ahmad, S. and Toboli A.S. (2011). Detection of metallo-betalactamase (MBL) producing *Pseudomonas aeruginosa* at a tertiary care hospital in Kashmir. Nordmann P, Naas T, Poirel L. Global spread of carbapenemase –Producing Enterobacteriaceae. *Emerging Infectious Diseases*, 17 (179), 1-8.
- Bonnedahl J, Drobni P, Johansson A, Hernandez J, Melhus A, Stedt J, *et al.* (2010). Characterization, and comparison, of human clinical and black-headed gull (*Larus ridibundus*) extended-spectrum beta-lactamase-producing bacterial isolates from Kalmar, on the southeast coast of Sweden. *Journal of Antimicrobial Chemotherapy*, 65, 1939.
- Bonten, M., Johnson, J. R., van den Biggelaar, A. H. J., Georgalis, L., Geurtsen, J., de Palacios, P. I., *et al.* (2021). Epidemiology of *Escherichia coli* Bacteremia: A Systematic Literature Review. *Clinical Infectious Diseases*, 72 (7), 1211–1219.
- Centre for Disease Control, CDC (2013). Guiding for Prevention of Surgical Site Infections. *Journal of Antimicrobial Chemotherapy*, 43: 447-458
- Cheesbrough, M. (2010). Biochemical tests to identify bacteria. In: *District laboratory practice in tropical countries* (part 2). Cambridge University Press, Cambridge, UK. Page, 71-76.
- Claude, V. (2013). Antibiotics for non humanuses. Department of Microbiology, Ayerst Research laboratories, Montreal Que., Canada.
- Clinical and Laboratory Standard Institute, CLSI (2018). Performance Standards for Antimicrobial Susceptibility Testing: Twenty-sixth Information Supplement CLSI document M100-S26. Wayne, P. A. 38:1-251
- da Costa P. M, Loureiro, L. and Matos, A. J. F.(2013). Transfer of multidrug resistant bacteria between intermingled ecological niches: the interface between humans, animals and the environment. *International Journal of Environtal Research and Public Health*, 10, 278.
- Dahiya, S., Singla, P., Chaudhary, U. and Singh, U. (2015). Carbapenemasea: A Review. *International Journal of Advanced Health Sciences*, 2(4), 11-17.

- Dyar, O. J., Hoa, N. Q., Trung, N. V., Phuc, H. D., Larsson, M., Chuc, N. T., *et al.* (2012). High Prevalence of Antibiotic Resistance in Commensal *Escherichia coli* Among Children in Rural Vietnam. *BMC Infectious Diseases*, 12, 92.
- Egbule, S. O. and Yusuf, I. (2019). Multiple Antibiotic Resistances in *Escherichia coli* Isolated from Cattle and Poultry Faeces in Abraka, South-South Nigeria. *Pertanika Journal of Tropical Agricultural Science*, 42 (2), 585 – 594.
- Egea, P., López-Cerero, L., Torres, E., Gómez- Sánchez, M. D. C., Serrano, L., Navarro Sánchez- Ortiz, M. D. (...) Pascual, A. (2012). Increased raw poultry meat colonization by extended spectrum beta-lactamase-producing *Escherichia coli* in the south of Spain. *International Journal of Food Microbiology*, 159 (2), 69–73.
- Ejikeugwu, C., Iroha, I., Duru, C., Ayogu, T., Orji, O., *et al.* (2016). Occurrence of metallo-beta-lactamase-producing enterobacteriaceae in abakaliki nigeria. *International Journal of Applied Pharmaceutical Sciences and Research*, 1, 70-75.
- Ejikeugwu, C., Ugwu, M., Iroha, I., Eze, P., Gugu, T., *et al.* (2014) Phenotypic detection of metallo-beta-lactamase (MBL) enzyme in Enugu, Southeast Nigeria. *America Journal Biology and Chemical Pharmaceutics Science*, 2, 2328-2814.
- Ferjani, S., Saidani, M., Maamar, E., Harbaoui, S., Hamzaoui, Z., Hosni, H., *et al.* (2018). *Escherichia coli* Colonizing Healthy Children in Tunisia: High Prevalence of Extra-Intestinal Pathovar and Occurrence of non-Extended- Spectrum- β -Lactamase-Producing ST131 Clone. *International Journal of Antimicrobial Agents*, 52 (6), 878–885.
- Hasan, B., Drobni, P., Drobni, M., Alam, M. and Olsen, B. (2012). Dissemination of NDM-1. *Lancet Infectious Diseases*, 12: 99.
- Hernandez J, Stedt J, Bonnedahl J, Molin Y, Drobni M, Calisto-Ulloa N, *et al.* (2012). Human-associated extended-spectrum beta-lactamase in the Antarctic. *Applied Environmental Microbiology*, 78, 2056.
- Hosuru S. S., Bairy, I., Nayak, N., Amberpet, R., Padukone, S., Metok, Y., Bhatta, D.R. and Sathian, B. (2020). Detection and Characterization of ESBL-Producing Enterobacteriaceae from the Gut of Healthy Chickens, *Gallus gallus domesticus* in Rural Nepal: Dominance of CTX-M-15-Non-ST131 *Escherichia coli* Clones. *PLoS ONE*, 15, e0227725.
- Islam, M. M., Islam, M. N. and Sharifuzzaman Fakhruzzaman, M. (2014). Isolation and identification of *Escherichia coli* and *Salmonella* from poultry litter and feed. *International Journal of Natural and Social Sciences*, 1, 1-7.
- Iyobe, S., Hisakanu, Y., Akio K., Ryoidi O., Hidero, K., Toshimitsu, K. and Mat Sushisa (2001). Plasmid Encoded, Metallo-Betalactamases, conferring Resistance to carbapenems, especially meropenem. *Journal of Antimicrobial Agents and chemotherapy*, 45 (5), 13-43.
- Johnson, J., Tchesnokova, V., Johnston, B., Clabots, C., Roberts, P. and Billing, M. (2013). Abrupt emergence of a single dominant multidrug-resistant strain of *Escherichia coli*. *Journal of Infectious Diseases*, 207(9), 19-28.

- Levin, B. R. (2001). Minimizing potential resistance: a population dynamics view. *Clinical Infectious Diseases*, 33, 161-169.
- Levy S. (1997). Antibiotic resistance: an ecological imbalance. Antibiotic resistance: origins, evolution, selection and spread. Ciba Foundation Symposium 207. Chichester: Wiley.
- Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E and Giske, C. G. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology Infection*. 2012; 18: E268-E281.
- Mahmoodi, F., Rezaatofighi, S. E., and Akhoond, M. R. (2020). Antimicrobial Resistance and Metallo-Beta-Lactamase Producing Among Commensal *Escherichia coli* Isolates From Healthy Children of Khuzestan and Fars Provinces; Iran. *BMC Microbiology*, 20 (1), 366.
- Martinez, J. L. (2008). Antibiotics and antibiotic resistance genes in natural environments. *Science*; 321: 365.
- Moore, P. R., Evenson, A., McCoy, E., Luckey, T. D., Elvehjem, C. A. and Hart, E. B. (2014). Antibiotic use in Animal Production. *Journal of Biological Chemistry*, 165 (437), 19-46.
- Nys, S., Okeke, I. N., Kariuki, S., Dinant, G. J., Driessen, C., and Stobberingh, E. E. (2004). Antibiotic Resistance of Faecal *Escherichia coli* From Healthy Volunteers From Eight Developing Countries. *Journal Antimicrobial Chemotherapy*. 54 (5), 952–955.
- Oli, A. N., Itumo, C. J., Okam, P. C., Ezebialu, I. U., Okeke, K. N., Ifezulike, C. C., Ezeobi, I., Emechebe, G. O., Okezie, U. M., Adejumo, S. A. and Okoyeh, J. N. (2019). Carbapenem-Resistant Enterobacteriaceae Posing a Dilemma in Effective Healthcare Delivery. *Antibiotics* , 8, Article No. 156.
- Russell, J. B. and Jarvis, G. N. (2001). Practical mechanisms for interrupting the oral-fecal lifecycle of *Escherichia coli*. *Journal Molecular Microbiology Biotechnology*, 3, 265-272.
- Sahoo, K. C., Tamhankar, A. J., Sahoo, S., Sahu, P. S., Klintz, S. R., and Lundborg, C. S. (2012). Geographical Variation in Antibiotic-Resistant *Escherichia coli* Isolates from Stool, Cow-Dung and Drinking Water. *International Journal Environmental Research and Public Health*, 9 (3), 746–759.
- Salysers, A. A., Gupta, A., and Wang, Y. (2004). Human Intestinal Bacteria as Reservoirs for Antibiotic Resistance Genes. *Trends in Microbiology*, 12 (9), 412–416.
- Shakya, P., Barrett, P., Diwan, V., Marothi, Y., Shah, H., Chhari, N., *et al.* (2013). Antibiotic Resistance Among *Escherichia coli* Isolates From Stool Samples of Children Aged 3 to 14 Years From Ujjain, India. *BMC Infectious Diseases*, 13, 477.
- Sjolund M, Bonnedahl J, Hernandez J, Bengtsson S, Cederbrant G, Pinhassi J, *et al.* (2008). Dissemination of multidrug-resistant bacteria into the Arctic. *Emerging Infectious Diseases*, 14, 70.
- Smith, D. L., Dushoff, J., Perencevich, E. N., Harris, A. D. and Levin, S. A. (2004). Persistent colonization and the spread of antibiotic resistance in nosocomial pathogens: resistance is a regional problem. *Proceedings of the National Academy of Science USA*, 101, 3709-3714.

- Thompson, K. S (2010). Extended– spectrum β –Lactamase, AmpC, and Carbapenemase Issues. *Journal of clinical Microbiology*, 48(4), 1019-1025.
- Toleman, M. A., Biedenbach, D., Bennett, D. M. C., Jones, R. N. and Walsh, T. R. (2005). Italian metallo– β –lactamases: a national problem? Report from the SENTRY Antimicrobial Surveillance Programme. *Journal of Antimicrobial Chemotherapy*, 55, 61-70.
- Tortola, M. T., Lavilla, S., Miro, E., Gonzalez, J. J., Larrosa, N., Sabate, M., Navarro, F. and Prats, G. (2005). First Detection of a Carbapenem–Hydrolyzing Metalloenzyme in Two Enterobacteriaceae Isolates in Spain. *Antimicrobial Agents and Chemotherapy*, 49(8), 3492-3494.
- Walsh, T. R., Toleman, M. A., Poirel, L. and Nordmann, P. (2005). Metallo- β -lactamases: the quiet before the storm? *Clinical Microbiology Review*, 18, 306–325.
- Zhong, Y. M., Liu, W. E., Meng, Q., and Li, Y. (2019). *Escherichia coli* O25b-ST131 and O16-ST131 Causing Urinary Tract Infection in Women in Changsha, China: Molecular Epidemiology and Clinical Characteristics. *Infect. Drug Resist.* 12, 2693–2702.