Asian Journal of Biochemistry, Genetics and Molecular Biology

Volume 13, Issue 1, Page 30-36, 2023; Article no.AJBGMB.95962
ISSN: 2582-3698

First Report Occurrence of *CIT* and *DHA* AmpC β-lactamase Gene in *Escherichia coli* and *Klebsiella pnuemoniae* from Clinical Sample in South Eastern, Nigeria

Peace Oluchi Akpu [a], Henrietta Onyinye Uzoeto [b], Ikemesit Udeme Peter [a,c*], Onyinye Lovette Nomeh [a], Agabus Chidiebube Nwuzo [a], Rebecca Chinenye Ogba [a,d] and Ifeanyichukwu Romanus Iroha [a]

[a] Department of Applied Microbiology, Faculty of Science, Ebonyi State University, Abakaliki, P.M.B. 53, Nigeria.
[b] Department of Microbiology, Faculty of Pure and Applied Science, Federal College of Dental Technology and Therapy, Trans Ekulu, P.M.B. 01473, Enugu, Nigeria.
[c] Department of Public Health, Faculty of Health Technology and Engineering, Federal College of Dental Technology and Therapy, Trans-Ekulu, P.M.B. 01473, Enugu, Nigeria.
[d] Department of General Studies, Science and Technology, Federal Polytechnic, Ohodo, P.M.B. 01801, Enugu, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Authors POA, RCO helped to conceptualized the data. Author POA did Data curation and Formal analysis. Authors HOU, ACN, IUP, IRI performed Methodology. Author POA did project administration. Authors IRI supervised the data and wrote the original draft. Authors IRI and IUP wrote, reviewed and edited the manuscript. All authors investigated the study, did literature searches and did data Validation and Visualization. All the authors reviewed and approved the final draft, and are responsible for all aspects of the work.

Article Information

DOI: 10.9734/AJBGMB/2023/v13i1285

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/95962

Original Research Article

Received: 28/10/2022
Accepted: 30/12/2022
Published: 03/02/2023

**Corresponding author: E-mail: ikemesitpeter@gmail.com;*

ABSTRACT

Background and Objectives: Over time, the enzymes AmpC β-lactamases have become more significant, due to their roles in antibiotic resistance among enterobacteriaceace especially in *Escherichia coli* and *Klebsiella pnuemoniae.* Due to increase multidrug resistant express by AmpC β-lactamases producing bacteria strain, the patients care in several hospital has been severely hampered. Hence, this study was designed to assess the occurrence of *CIT* and *DHA* AmpC β-lactamase gene in *Escherichia coli* and *Klebsiella pnuemoniae* from clinical sample in south eastern, Nigeria

Methodology: This study was conducted over an 8-month period on sixteen (16) non-repetitive clinical isolates of *Escherichia coli* and *Klebsiella pnuemoniae* collected from medical microbiology laboratory unit of Alex Ekweume Federal University Teaching Hospital in Abakaliki, Nigeria. The isolates were further identified using Standard microbiological Techniques and screened for cefoxitin resistance using a disc diffusion assay, followed by phenotypic tests using phenyl boronic acid assays for confirmation of AmpC β-lactamases production. *Escherichia coli* and *Klebsiella pneumoniae* strains were further screen for AmpC β-lactamase *CIT* and *DHA* genotype by polymerase chain reactions

Result: Of the sixteen (16) confirmed phenotypic AmpC β-lactamase producing bacteria, 100% of the AmpC β-lactamase genes (*DHA* and *CIT*) were detected in *E. coli* from wound and urine samples from both male and female patients**.** The overall proportion of AmpC β-lactamases gene in *Klebsiella pneumoniae* were *DHA* (100 %) and *CIT* (100 %)*,* in both male and female.

Conclusion: This study indicate the occurrence of *CIT* and *DHA* AmpC genotype. The detection of AmpC β-lactamases in this study is of clinically importance as such bacteria are often MDR. Thus, being aware of the presence of AmpC β-lactamase-producing bacteria could be very beneficial for achieving more accurate epidemiological results as well as controlling their spread, while surveillance is required to track any further dissemination and emergence of other AmpC β-lactamase genotypes.

Keywords: AmpC β-lactamases; Escherichia coli; Klebsiella pneumoniae; CIT; DHA.

1. INTRODUCTION

Escherichia coli and *Klebsiella pnuemoniae* are members of the enterobacteriaceae family. This two medical important bacteria genera are associated with both nosocomial and opportunistic infections. *Escherichia coli* and *Klebsiella pneumoniae* are the common etiologic agent of various human disease such as infantile enteritis, septicemia, urinary tract infections, meningitis and bacteremia [1, 2]. Also, they are implicated in diseases and infections among patients in the hospital with immunodeficiency and underlying conditions such as chronic pulmonary disorders and diabetes [1, 2]. In recent time, the action of several antibiotic in the treatment of these bacterial infections has been stall or truncated due to the production of β-lactamase especially AmpC β-lactamase. This enzymes are distinguished by their ability to inactivate cephamycins as well as other extended-spectrum cephalosporins and their resistance to clavulanic acid. The occurrence of AmpC β-lactamase resistant determinant has severely hampered patients care as they are often multidrug resistant. Over a decade, there has been an upsurge in the global prevalence of AmpC β-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* with significant morbidity and mortality rate among patients [2,3,4,5,6,7,8,9,10].

The AmpC β-lactamase genotypes may be plasmids or chromosomal mediated but based on their genetic similarities to species specific AmpC β-lactamase, plasmid variants groups include *Morganella morganii DHA* variants, *CIT* variants (*CMY*-2 types) from *Citrobacter freundii*, *Hafnia alvei*, *ACC* variants from *Aeromonas* species and the *Enterobacter* species *EBC* variants (*ACT*-1 type, *MIR*-1) are widely dissemination in different geographical settings [2,6,10,11,12] but the knowledge of some AmpC β-lactamase genotype circulating this area remain unknown. The genotypes *FOX*, *ACC*, *EBC*, *DHA*, *MOX*, *CIT* and *CMY* are the most frequent AmpC β-lactamase reported elsewhere [2, 6,10] but there has been no clinical research on the occurrence of AmpC β-lactamase genotype to date in Abakaliki, South eastern, Nigeria. Accurate detection of AmpC genotype (*CIT* and *DHA*) may not only deemed essential for managing the health of patients

suffering from *Escherichia coli* and *Klebsiella pnuemoniae* infections but is also helpful for analyzing the regional distribution of AmpC β-lactamase genotype through epidemiological research.

2. MATERIALS AND METHODS

2.1 Bacterial Identification

The study was approved by the Ethical Research committee of Ebonyi State Ministry of Health, Abakaliki with approval number SMOH/ERC/042/21 and was carried out in line with the Declaration of Helsinki [13,14]. This study was performed over an 8-months period on sixteen (16) non-repetitive clinical isolates of *Escherichia coli* and *Klebsiella pnuemoniae* collected from medical microbiology laboratory unit of Alex Ekweume Federal University Teaching Hospital, Abakaliki in southeastern Nigeria. It is located at 6.32^{o}N latitude and 8.12^{o}E longitude and is situated at an elevation of 117 meters above sea level. The bacteria isolates were further identified using Standard microbiological Techniques [15,16]. The confirmed isolates were stored until further test [15].

2.2 AmpC Production Testing

Escherichia coli and *Klebsiella pnuemoniae* isolates were initially screened for the potential of AmpC β-lactamases production using a cefoxitin disk (30 *µg*) (Oxoid, UK) placed on sterile solidified Mueller-Hinton agar (Merck Co., Germany) containing the bacteria isolates [17]. After 24hours of incubation, inhibition zone diameter of greater than 18 mm were phenotypically inferred as AmpC β-lactamases producers and were subjected to the confirmatory phenotypic test using inhibitor-based method on a disk containing boronic acid [18]. This was performed as follows: a standardized 0.5 McFarland turbidity suspension of the test isolates was evenly spread on a sterilized Mueller-Hinton agar plates. The test was performed by placing two disks of cefoxitin (30 *µ*g) (Oxoid, UK) on the sterile agar surface, one with and one without phenylboronic acid (400 g) (SIGMA-ALDRICH, Co., U.S.A) and incubated for 24hours. After overnight incubation, growth of inhibition zone of 5 mm or greater around the antibiotic with phenylboronic acid when compared to the disk containing only cefoxitin, the isolate was considered an AmpC producer [17,18].

2.3 Extraction of Bacterial DNA

All DNA from pure culture phenotypic AmpC β-lactamases producing *Escherichia coli* and *Klebsiella pneumoniae* were extracted using the ZR fungal/bacterial DNA MiniPrep kit [14,19]. The AmpC β-lactamase encoding genes (*CIT* and *DHA*) were separately amplified using a Mastercycler ®X50 thermal cycler (Azure Biosystem, Dublin, CA) utilizing the appropriate primers from Invitrogen, U.S. A for *CIT* gene F-TGGCCAGAACTGACAGGCAAA; R- TTTCTCCTGAACGTGGCTGGC; *DHA* gene; F- AACTTTCACAGGTGTGCTGGGT; R- CCGTACGCATACTGGCTTTGC [20]. The amplification process was performed as described by Pérez-Pérez and Hanson [20]. The PCR products were separated on 1.5% agarose gel prepared in 1X TBE (Tris/Boric/EDTA) buffer and visualized under UV trans-illuminator using a gel documentation system.

3. RESULTS

3.1 Occurrence of AmpC β-lactamase Genes in Isolates of *E. coli and K. pneumoniae*

Of the sixteen (16) confirmed phenotypic AmpC β-lactamase producing bacteria, 100 % of the following AmpC β-lactamase genes (bla_{DHA}, bla_{CIT}) were detected in *E. coli* from urine and wound samples of both male and female patients as shown in Table 1**.** The overall proportion of AmpC β-lactamases gene were bla_{DHA} (100 %) and bla_{CIT} (100 %)*,* in both male and female as shown in Table 2.

4. DISCUSSION

AmpC β-lactamases gene amplified by PCR revealed high occurrence rate of bla_{DHA} (100 %)*,* and bla_{CIT} (100 %)*.* Our findings reiterate with report from other studies; a study in Iran reported *CIT* and *DHA* as the most common AmpC genotype in *E. coli* [21]. Earlier report in Tehran Northern Iran also showed abundance of bla_{DHA} and bla_{CIT} in *Klebsiella* species [22]. Also, *CIT* and *DHA* AmpC β-lactamases has been found in Bahrain in *E. coli* and *K. pnuemoniae* resistant to cefoxitin [10]. However, geographic diversity has been discovered through studies conducted in various parts of the world on the occurrence of AmpC β-lactamases genotype. Earlier studies has shown widespread of bla_{CIT} subtype in Ireland, United Kingdom, Canada and United State of America [23, 24] while in India bla_{DHA} was identified in *E. coli* 38.0 % and *Klebsiella* species 46.7 % [25].

Table 1. Occurrence of AmpC β-lactamase genes in *E. coli*

Clinical Sample	Gender	Bacteria coding	*blaDHA* (%)	*blaCIT* (%)
Urine	Male	EC1	1(12.5)	1(12.5)
		EC2	1(12.5)	1(12.5)
	Female	EC3	1(12.5)	1(12.5)
		EC4	1(12.5)	1(12.5)
Wound swab	Male	EC5	1(12.5)	1(12.5)
		EC6	1(12.5)	1(12.5)
	Female	EC7	1(12.5)	1(12.5)
		EC8	1(12.5)	1(12.5)
Total		**(n=8)**	**8(100)**	**8(100)**

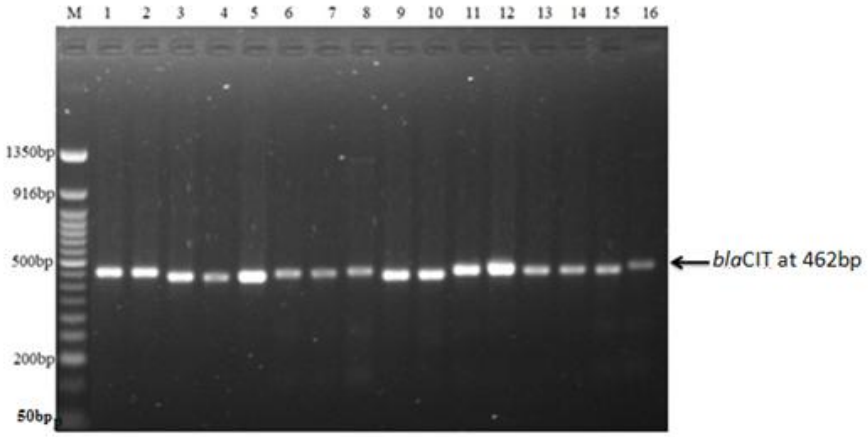

Plate 1. Gel image amplification of *bla*CIT at about 462bp. A 50bp ladder was used to estimate the base pair size of the amplicons. Lane M – 50bp molecular marker, Lane 1 – 8 = *E. coli*, Lane 9 – 16 = *K. pneumoniae* isolates

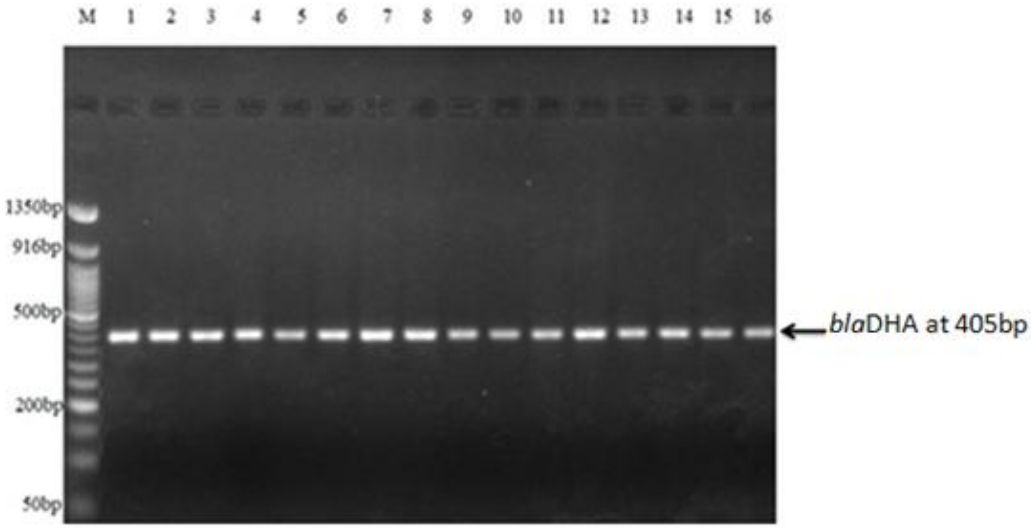

Plate 2. Gel image amplification of *bla*DHA at about 302bp. A 50bp ladder was used to estimate the base pair size of the amplicons. Lane M – 50bp molecular marker, Lane 1 – 8 = *E. coli*, Lane 9 – 16 = *K. pneumoniae* isolates

Table 2. Occurrence of AmpC β-lactamase genes in *K. pneumoniae*

Clinical Sample	Gender	Bacteria coding	*DHA* (%)	*blaCIT* (%)
Urine	Male	K9	1(12.5)	1(12.5)
		K10	1(12.5)	1(12.5)
	Female	K11	1(12.5)	1(12.5)
		K12	1(12.5)	1(12.5)
Wound swab	Male	K13	1(12.5)	1(12.5)
		K14	1(12.5)	1(12.5)
	Female	K15	1(12.5)	1(12.5)
		K16	1(12.5)	1(12.5)
Total		**(n=8)**	**8(100)**	**8(100)**

The origin of bla_{DHA} as plamid-borne AmpC genotype has been linked to acquisition of chromosomal AmpC β-lactamases gene from *Morganella Morganii's* [26]. In a study conducted in the year 2004 and 2008, it was discovered that pathogenic bacteria producing bla_{DHA} showed high mortality among infected patients over those organism harboring bla_{CMY-1} gene [27,28] and has raise concerns about the dissemination AmpC β-lactamases mediated by an inducible plasmid. According to another study, all *Escherichia coli* isolates tested positive for the bla_{CIT} family [29,30]. As noted in this study, *DHA* and *CIT* AmpC β-lactamases gene were commonly identified, indicating rapid plasmid expression and gene dissemination in recent times in the study area.

In spite of the fact that they seems to be a variation in the occurrence of *DHA* and *CIT* reported in this study over other studies. Such discrepancies could be linked to the length of the study which have an impact on the prevalence of the gene, the geographical area, number of samples, the bacteria isolate evaluated and the AmpC β-lactamases genotype detected. As such it will be difficult to comparing the occurrence of AmpC β-lactamases gene across studies.

According to our findings, the *CIT* and *DHA* gene appears to be an important resistant determinant in plasmid mediated AmpC β-lactamases producing bacteria dissemination. The detection of AmpC β-lactamases in this study is of clinically importance, as such bacteria are often MDR due to mutations that lower the production of porin, and can lead to resistance in such strains towards broader cephalosporin, β-lactamase inhibitors and other antibiotic class.

5. CONCLUSION

The study provides data on the first reported occurrence of *DHA* and *CIT* types *AmpC* isolates in southeastern Nigeria. The dissemination of *DHA* and *CIT* AmpC β-lactamases genes beyond the hospital or across the country via conjugation may become a significant public health issue. As a result of identifying *DHA* and *CIT* types of AmpC β-lactamases may assist physicians in prescribing suitable antibiotics that will reduce selective pressure that heightens antibiotic resistance among bacteria isolates while surveillance is recommended to track any further dissemination and emergence of other AmpC β-lactamase genotypes.

6. LIMITATIONS

The lack of data on the sequencing of AmpC cluster genes was one of the study's limitations. Furthermore, due to a lack of funding, only the presence of *CIT* and *DHA* AmpC genes was investigated, while other AmpC genes, chromosomal hyperproduction or purine loss mutations, were not investigated

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

In compliance with international standard or university standard written ethical approval has been collected and preserved by the author (s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kaye KS, Pogue JM. Infections caused by resistant Gram-negative bacteria: Epidemiology and management.

Pharmacotherapy: J Human Pharm Drug Ther. 2015;35(10):949-962

2. Golsha R, Maryam M, Nazanin R, Mina EZ. Frequency of beta-lactamase antibiotic resistance genes in *Escherichia Coli* and *Klebsiella pneumoniae*, Gorgan, Iran. Ethiop J Health Sci. 2021;31(3):663.
3. Rodríguez-Guerrero E, Callejas-Rodelas JC, Navarro-Marí JM, Gutiérrez-Fernández J. Systematic review of plasmid AmpC type resistances in *Escherichia coli* and *Klebsiella pneumoniae* and preliminary proposal of a simplified screening method for AmpC. Microorganisms. 2022;10:611-612.
4. Singhal S., Mathur T, Khan S. Evaluation of methods for AmpC *β*-lactamase in gram-negative clinical isolates from tertiary care hospitals. Indian J Medical Microbiol. 2005;23(2):120–124.
5. Livermore DM. Current epidemiology and growing resistance of gram-negative pathogens. Korean J Internal Med. 2012;27(2):128–142.
6. Sadeghi H M, Ebrahim-Saraie S, Mojtahedi A. Prevalence of ESBL and AmpC genes in *E. coli* isolates from urinary tract infections in the north of Iran. New Microbes and New Infections. 2021;45:47-60
7. Martinez‐Martinez L, Conejo MC, Pascual A, Hernandez‐Alles S, Ballesta S, Ramírez De Arellano‐Ramos E, et al. Activities of imipenem and cephalosporins against clonally related strains of *Escherichia coli* hyperproducing chromosomal beta‐lactamase and showing altered porin profiles. Antimicrob Agents Chemother. 2000;44:2534‐6.
8. Park SD, Uh Y, Lee G, Lim K, Kim JB, Jeong SH. Prevalence and resistance patterns of extended-spectrum and AmpC β-lactamase in *Escherichia coli, Klebsiella pneumoniae*, *Proteus mirabilis*, and *Salmonella* serovar *stanley* in a Korean tertiary Hospital. J Clin Microbiol. 2010;118(10):801–808.
9. Jameel NA, Ejaz H, Zafar A, Amin H. Multidrug resistant AmpC β-lactamase producing *Escherichia coli* isolated from a paediatric hospital. Pak J Med Sci. 2014;30(1):181-184.
10. Joji RM, Al-Mahameed AE, Al Jishi T, Fatani DI, Saeed NK, Jaradat A, et al. Molecular detection of plasmid-derived AmpC β-lactamase among clinical strains of *Enterobacteriaceae* in Bahrain. Ann Thorac Med. 2021;16:287-93.
11. Ejikeugwu C, Esimone C, Iroha IR, Adikwu M. First detection of FOX-1 AmpC β-lactamase gene expression among *Escherichia coli* isolated from abattoir samples in Abakaliki, Nigeria. Oman Medical Journal. 2018;33(3):243-249.
12. Hanson ND. AmpC beta‐lactamases: what do we need to know for the future? J Antimicrob Chemother. 2003;52:2‐4.
13. World Medical Association (WMA) Declaration of Helsinki. Ethical principles for medical research involving human subjects. Note of Clarification on Paragraph 30 by the WMA General Assembly, Tokyo. 2004;9-10.
14. Peter IU, Emelda NC, Chukwu EB, Ngwu JN, Uzoeto HO, Moneth EC Stella AO, Edemekong CI, Uzoamaka EP, Nwuzo AC, Iroha IR. Molecular detection of bone sialoprotein-binding protein (*bbp*) genes among clinical isolates of methicillin resistant *Staphylococcus aureus* from hospitalized orthopedic wound patients. Asian J Orthopaedic Res. 2022,8(3):1-9.
15. Iroha IR, Orji JO, Onwa NC, Nwuzo AC, Okonkwo EC, Ibiam EO, Nwachi AC, Afuikwa FN, Agah VM, Ejikeugwu EPC, Agumah NB, Moses IB, Ugbo E, Ukpai EG, Nwakaeze E A, Oke B, Ogbu L and Nwunna E. Microbiology practical handbook. (Editor; Ogbu. O), 1st Edition. Charlieteximage Africa (CiAfrica Press). 2019:344.
16. Edemekong, CI, Iroha IR, Thompson, MD, Okolo, IO., Uzoeto HO, Ngwu JN, Mohammed ID, Chukwu EB, Nwuzo AC, Okike BM, Okolie SO, Peter IU. Phenotypic characterization and antibiogram of non-oral bacteria isolates from patients attending dental clinic at Federal College of Dental Technology and Therapy Medical Center Enugu. Int J Pathog Res. 2022;11(2):7-19.
17. Polsfuss S, Bloemberg GV, Giger J, Meyer V, Böttger EC, Hombach M. Practical approach for reliable detection of AmpC beta-lactamase-producing enterobacteriaceae. J Clin Microbiol. 2011;49(8):2798-2803.
18. Coudron PE. Inhibitor-based methods for detection of plasmidmediated AmpC beta-lactamases in Klebsiella spp., *Escherichia coli*, and *Proteus mirabilis*. J Clin Microbiol. 2005;43:4163−7.

19. Edemekong CI, Uzoeto HO, Mbong EO, Ikusika BA, Didiugwu, CM, Ngwu JN, NseAbasi PL, Ntekpe ME, Mohammed ID, John-Onwe BN, Alagba EE, Obodoechi IF, Joseph OV, Ogbonna IP, Ubom IJ, Peter IU. Molecular characterization and bioassay of soil *Actinomycetes* strains on multidrug resistant bacteria. IOSR J Biotechnol Biochem. 2022;(1):6-11.
20. Pérez-Pérez FJ, Hanson ND. Detection of plasmid-mediated *AmpC* Beta-lactamase genes in clinical isolates by using multiplex PCR. J Clin Microbiol. 2002;40: 2153–62.
21. Maleki A, Khosravi A, Ghafourian S, Pakzad I, Hosseini S, Ramazanzadeh R, et al. High prevalence of AmpC β-Lactamases in clinical isolates of *Escherichia coli* in Ilam, Iran. Osong Public Health Res Perspectives. 2015;6(3):201–4.
22. Ghanavati R, Darban-Sarokhalil D, Navab-Moghadam F, Kazemian H, Irajian G, Razavi S. First report of coexistence of AmpC betalactamase genes in *Klebsiella pneumoniae* strains isolated from burn patients. Acta Microbiologica et Immunologica Hungarica. 2017;64(4):455–62.
23. Oliveira C, Amador P, Prudêncio C, Tomaz CT, Tavares‐Ratado P, Fernandes R. ESBL and AmpC‐lactamases in clinical strains of *Escherichia coli* from Serra da Estrela, Portugal. Medicina (Kaunas). 2019;55:272.
24. Woodford N, Reddy S, Fagan EJ, Hill RL, Hopkins KL, Kaufmann ME. Wide geographic spread of diverse acquired AmpC beta‐lactamases among *Escherichia coli* and *Klebsiella* species in the UK and Ireland. J Antimicrob Chemother; 2007;59:102‐5.
25. Mohamudha PR, Harish BN, Parija SC. Molecular Description of Plasmid-mediated AmpC beta-lactamases among nosocomial isolates of *Escherichia coli* and *Klebsiella pneumoniae* from six different hospitals in India. Indian J Medl Res. 2012;135:114–9.
26. Verdet C, Arlet G, Barnaud G, Lagrange PH, Philippon A. A novel integron in *Salmonella enterica* serovar enteritidis, carrying the *bla*(DHA-1) gene and its regulator gene *amp*R, originated from *Morganella morganii*. Antimicrob Agents and Chemother. 2000;44(1):222–225.
27. Pai H, Kang CI, Byeon JH. Epidemiology and clinical features of bloodstream infections caused by AmpC-type- β-lactamase-producing *Klebsiella pneumoniae*. Antimicrob Agents and Chemother. 2004;48(10):3720–3728.
28. Moland ES, Kim SY, Hong SG, Thomson KS. Newer beta-lactamases: Clinical and laboratory implications, part I." Clin Microbiol Newsletter. 2008;(30):71–77.
29. Dallal MS, Sabbaghi A, Aghamirzaeie HM, Lari AR, Eshraghian MR, Mehrabad JF. Prevalence of AmpC and SHV beta-lactamases in clinical isolates of *Escherichia coli* from Tehran Hospitals. Jundishapur J Microbiol. 2013;6(2):176-80.
30. Thoms Rodriguez CA, Dawson F, Cameron J, Seah C, Reid M, Melano RG, Gossell-Williams M. Prevalence and distribution of AmpC beta-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* isolates obtained from urine samples at a tertiary care hospital in the Caribbean. Front. Cell. Infect. Microbiol. 2022;12:1015-633.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/95962