DOI: https://doi.org/10.54393/pbmj.v5i4.258

PAKISTAN BIOMEDICAL JOURNAL

https://www.pakistanbmj.com/journal/index.php/pbmj/index

Volume 5, Issue 4 (April 2022)

Original Article

Study of Application of E-Test for the Detection of Beta-Lactamase Producing Bacteria

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ARTICLE INFO

Key Words:

Antibiotic Resistance, E- Test, Beta- Lactamases, *E. coli, Enterobacter, Klebsiella pneumonia*

How to Cite:

Saleem, M.., Azam, Z.., Kausar, S., Azam, H. A. ., Atiq, A. ., Akram, J. ., Jameel, L. ., Mehmood, Z. ., & Hayee, S. . Study of Applications of E-Test for the Detection of Beta-Lactamase Producing Bacteria. Pakistan BiomedicalJournal,5(4).https://doi.org/10.54393/pbmj.v5i4.258

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Received Date: 13th Feb, 2022
Acceptance Date: 20th April, 2022
Published Date: 30th April, 2022

ABSTRACT

The term antimicrobial resistance refers to the ability to resist the effects of drugs formally used to treat them and this term relates only to bacteria becoming resistant. Microorganisms which are resistant to multiple drugs are known as multidrug resistant bacteria. **Methods:** Most of the experimental work to study the application of E-Test for detection of lactamase producing bacteria was carried out at Pathology Laboratory of Sir Ganga Ram Hospital Lahore. While the remaining research work was done in Microbiology Laboratory of Govt. Post Graduate Islamia College Cooper Road Lahore during the study period from December 2019 to March 2020. Total 60 samples of different patients were collected from Sir Ganga Ram Hospital Lahore and most of the samples were urine (n=25), followed by Blood (n=14), Pus (n=14), and sputum (n=7). Oxidase, indole, citrate utilization, sugar fermentation (Kligler iron agar medium) and urease tests were performed for the identification bacterial strains. **Results:** In all of 60 samples frequency of occurrence of *E. coli*, *Enterobacter* and *Klebsiella* strains were 59%, 23%, 18% respectively. Most of them (n=36) were ESBLs positive and about (n=24) were ESBLs negative and their percentage were 60% and 40% respectively. Extended spectrum β-lactamase (ESBL) producing strains of *Enterobacteriaceae* have now become as a significant issue in hospitalized and community patients. These microorganisms are liable for many diseases, for example, urinary tract infection, septicemia, hospitalized-acquired pneumonia, intra-abdominal abscess, brain abscess and device related infections. **Conclusions:** The frequency of ESBL producing bacteria in most hospitals is very high especially in the hospitals where broad spectrum antibiotics are generally recommended. Among gram negative bacteria, the emergence of resistance to expanded spectrum cephalosporins has been a major concern. Many of ESBL producing bacteria showed multidrug resistance.

INTRODUCTION

Antibiotic resistance is an issue of profound logical concern both in emergency clinic and community settings. Fast discovery in clinical research facilities is basic for the reasonable acknowledgment of antimicrobial resistant organism. The production of extended spectrum β-lactamases (ESBLs) is a critical obstruction system that hinders the antimicrobial treatment of diseases brought about by Enterobacteriaceae and is a genuine danger to the present accessible antibiotic arsenal. ESBLs are characterized into a few groups as per their amino-acid sequence homology [1]. Epsilometer test (E-Test) is an outstanding gradient approach for the assurance of antimicrobial resistance. The E-Test has been developed for the immediate degree of antimicrobial susceptibility of microorganisms [2]. The Minimum Inhibitory Concentration (MIC) can be controlled by utilizing E-Test which is a research center test technique. E-Test is utilized to check the weakness of specific strain of microbes or growth to the activity of specific antibiotics [3]. The E-Test strips are non-pours and weaken on one side with a preset and controlled slope of 15 antibiotic fixations. The opposing side of nylon strips has a MIC scale engraved on it. In excess of 100 antimicrobial references are existing for E-test, which can be characterized into 4 areas that are antibiotics

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agents, antifungal enemy of mycobacterial and resistance phenotype testing [4]. Practicing an antibiotic to test the susceptibility of a life form (microbes), it gives the concentrations of antibiotics needed to inhibit the microscopic organisms being tested [5]. The MIC of organisms is the least amount of antimicrobial that can harm a living thing. If the MIC is necessary and agar lawn plates are already being established, or if the ability to read broth microdilution plates is unavailable in the research facility, the antibiotic strip technique can be used. Antimicrobial strips are available from a variety of manufacturers, including bioMérieux [6]. For antimicrobial susceptibility, the E-test is a quantitative technique that uses a combination of dilution and diffusion principles. When an E-test strip is placed on an inoculated culture plate, antibiotic drugs are quickly transported from the plastic transporters surface to the surface of the agar. Bacterial growth following incubation is visible, with a symmetrical inhibitory ellipse along the length of the strip [7]. Where the elliptical edge touches the strips, the MIC value is taken from the scale in terms of ml [8]. Despite the fact that carbapenemases are usually resistant to beta lactamase, bacteria generate enzymes that confer multi-resistance to -lactam antibacterial drugs, cephalosporins, cephamycins, and carbapenems (Ertapenem). Antibiotic resistance is caused by beta-lactamase, which breaks down the structure of antibiotics [9]. A four-atom ring known as a lactam is present in all of these antibiotics' molecular structures. Lactamase tears the beta-lactam ring open during hydrolysis, destroying the molecule's antibacterial properties. Antibiotics known as beta-lactams are frequently used to diagnose a wide spectrum of Gram-positive and Gram-negative bacteria. Bacilli organisms secrete beta-lactamases, especially when antibiotics are present in the environment [10].

METHODS

Most of the experimental work to study the application of E-Test for detection of lactamase producing bacteria was carried out at Pathology Laboratory of Sir Ganga Ram Hospital Lahore. While the remaining research work was done in Microbiology Laboratory of Govt. Post Graduate Islamia College Cooper Road Lahore during the study period from December 2019 to March 2020. Total 60 samples of different patients were collected from Sir Ganga Ram Hospital Lahore and most of the samples were of the urine (n=25), followed by Blood (n=14), Pus (n=14), and sputum (n=7). The materials were cultured on a variety of bacteriological medium, including MacConkey's Agar, Blood Agar, Chocolate Agar, and CLED. All samples were cultivated according to the manufacturer's instructions and incubated at 37°C until enough growth was achieved. After incubation for 24 hours the colonies of bacteria were picked and dissolved the bacterial colonies in saline solution which were in test tube. A cotton swab was dipped in the saline which contains bacteria. The cotton swab was removed from saline solution in such way that the extra saline remains in test tube [19]. The inoculums were applied with a sterile cotton swab on each agar plate using a fresh swab for each plate. The antibiotic carriers' strips were then applied in radial pattern on each plate. The plates were incubated for 24 hours at 37oC in an anaerobic chamber. Antibiotic sensitivity testing was performed on samples with distinctive staining and morphological properties. According to the clinical laboratory standards institute's standards, zones of inhibition were assessed and classified as sensitive or resistant [39]. The generation of beta lactamase by infection-producing isolates was isolated, characterized, and confirmed using the Standard Operating Procedure established by the health promotion board [40]. Gram staining and a biochemical test were used to identify each strain. Gram staining was used to identify Gram negative bacteria. Gram positive bacteria were detected as purple colonies, while gram negative bacteria were identified as pink colonies. Standard biochemical assays such as the indole test, urease test, Kliglar Iron agar medium, citrate utilization test, and oxidase test were used to further identify bacterial strains, according to clinical microbiology guides [20,38,]. The ellipse of inhibition created around different strips was used to check bacterial strains' resistance to various antibiotics. Multi-drug resistant (MDR) bacteria are those that are resistant to two or more antibiotics (carbapenems, fluoroquinolones, Penicillins/cephalosporins, and aminoglycosides). The Clinical Laboratory Standards Institute (CLSI) was used to evaluate the results.

RESULTS

Gram-negative bacteria have developed sophisticated mechanisms of resistance to most strong antibiotics over last two decades. This has been a global issue that the World Health Organization has identified as one of the greatest threats to human health. The overuse and misuse of these treatments, as well as the pharmaceutical company's lack of novel antimicrobials, are largely to blame for the current issue. This resulted in a longer trip to the hospital, a significant rise in costs, and a rise in death rates. It makes urological operations more dangerous and complicated. In both community and hospital settings, urinary tract infection is among the most opportunistic infections. Gram-negative bacteria are the most common cause. We are concentrating on the most prevalent and difficult group of Gram-negative bacteria, ESBL producers, in our work. Between December 2019 and March 2020, 60 clinical specimens of urine, pus, blood, and sputum were collected from Sir Ganga Ram Hospital in Lahore. The

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bacterial strains were identified using oxidase, indole, citrate utilization, sugar fermentation (Klingler iron agar medium), and urease tests. The frequency of E. coli, Enterobacter, and Klebsiella bacteria was 59 in all of the samples indicated above. Total (n=60) samples were collected from Sir Ganga Ram Hospital Lahore. Most of them (n=36) were ESBLs positive and about (n=24) were ESBLs negative and their percentage were 60% and 40% respectively (Figure 1). In total 60 collected samples (n=25) were of urine, of which 64% were ESBLs producers and 36% were non-producers. Total (n=14) samples of blood were collected of which 57% are ESBLs producers and about 43% were non-producers. Total (n=14) samples of pus were collected of which about 64% were ESBLs positive while 36% were negative. There were (n=7) out of (n=60) samples were of sputum and their percentage is about 43% and 57% are ESBLs producers and non-producers respectively (Figure 2). It was found that E. coli was 64%, 57%, 57% and 42% in samples of urine, blood, pus and sputum respectively. The results were found that 20%, 43%, 7% and 29% Enterobacter spp. were in samples of urine, blood, pus and sputum respectively (Figure 3). It was estimated that about 16%, 0%, 36% and 29% of Klebsiela spp. were found in samples of urine, blood, pus and sputum respectively. Total 60 different clinical samples were collected from which 36 was ESBLs positive and 24 were ESBLs negative (Table 1).

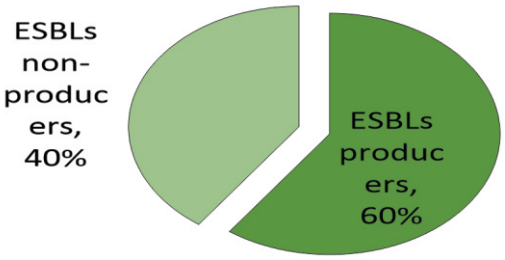

Figure 1: prevalence of ESBLs producers and ESBLs non-producers

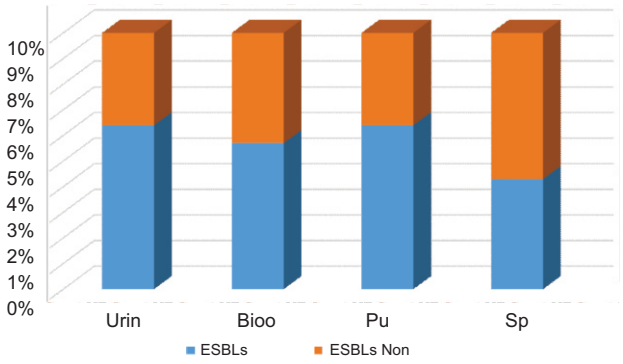

Figure 2: Prevalence of ESBLs producers and ESBLs non-producer in different clinical samples

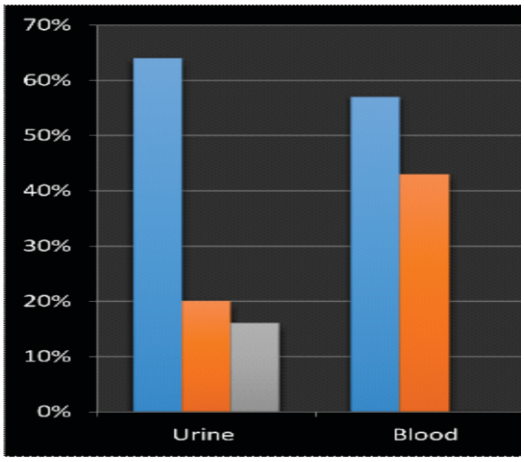

Figure 3: Prevalence of different bacterial strains in different clinical samples

Samples	samples	ESBLs +	ESBL
Urine	25	16	9
Pus	14	9	5
Blood	14	8	6
Sputum	7	3	4
Total	60	36	24

Table 1: Prevalence of ESBLs + and ESBLs – Bacteria in different clinical samples

DISCUSSION

Bacteria keep on assuming a significant part as a reason for medical care related diseases. Bacterial antibiotic resistance has become a significant clinical worry all through the Globe [18, 27, 28]. The use of second or third generation cephalosporins has subsequently resulted in the discovery of gram-negative bacteria that are resistant to extended spectrum cephalosporins. The creation of expanded spectrum -lactamases is the source of this hostility [11, 29, 31]. Clinical treatment failures occur frequently, especially when the inappropriate antibiotic medication is used to treat infections caused by ESBL-producing microorganisms. When airborne contaminants with ESBL-producing microscopic organisms can be identified based on a patient's clinical features, this may lead to a more accurate antibiotic selection and enhanced illness outcomes [12, 30, 32, 33]. Blood Stream Infections (BSI) brought about by extended spectrum β-lactamase (ESBL) producing living beings notably increment the paces of treatment failure and death [13,34, 35] Urinary Tract Infections (UTI) remains the basic infections analyzed in outpatients just as in hospitalized patients [36]. Current information on antimicrobial susceptibility pattern of urine microorganisms is required for proper treatment. Extended spectrum beta lactamases (ESBL) hydrolyze expended spectrum cephalosporins like ceftazidime,

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cefotaxime which are utilized in the treatment of UTI. ESBL producing microscopic organisms may not be detectable by routine disk diffusion susceptibility test [37] prompting improper utilization of antibiotics and treatment failure. Very little data on ESBL producing bacteria causing UTI is accessible from India [17, 21, 22]. An exertion was made to consider the ESBL producing urine pathogens and furthermore the susceptibility examples of ESBL and non-ESBL makers [14]. In most hospitals [23] the prevalence of ESBL-producing microorganisms is extremely high [24] particularly in those where broad-spectrum antibiotics are commonly prescribed. The evolution of resistance to expanded range cephalosporins among gram negative has become a major source of worry [25]. Many Extended - spectrum beta bacteria were shown to be multidrug resistant. Antibiotic resistance has emerged in bacteria that do not manufacture AmpC enzymes naturally (K. pneumoniae) due to the development of TEM- or SHV-type ESBLs [26].

CONCLUSIONS

Because of the constraints provided by high levels of resistance to multiple antibiotics, treating infections caused by MBL strains is indeed a therapeutic challenge. The synthesis of numerous such enzymes by MBL positive bacteria could be indicated by their resistance to a broad range of antibiotics. Longer hospital stays and prolonged antibiotic treatment (particularly carbapenems) are likely to be the primary risk factors for the establishment of gram-negative Metallo-beta-lactamase producers. Most practitioners in poorer nations like Pakistan are simply ignorant, which is exacerbated by inadequate infection control measures and the over-the-counter availability of medicines. In all cases where a -lactamase-producing bacteria is anticipated, however, the choice of a suitable lactam antibiotic should be carefully evaluated before therapy. In particular, choosing appropriate β-lactam antibiotic therapy is of upmost importance against organisms with inducible β-lactamase expression.

REFERENCES

[1] Qureshi M, Asif N, Baig S. Evaluation of extended spectrum β-lactamase mediated resistance in Escherichia coli and Klebsiella in urinary tract infection at a tertiary care hospital. Biomedica. 2013, 29(2):78-81.

[2] John C, Christenson Ryan F, Relich. Principles and Practice of Pediatric Infectious Diseases.5th ed. Elsevier Health Science.2018: 201-210.

[3] Shaikh S, Fatima J, Shakil S, Rizvi SM, Kamal MA. Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. Saudi J Biol Sci. 2015,22(1):90-101. doi: 10.1016/j.sjbs.2014.08.002.

[4] Alsterlund R, Carlsson B, Gezelius L, Haeggman S, Olsson-Liljequist B. Multiresistant CTX-M-15 ESBL-producing Escherichia coli in southern Sweden: Description of an outbreak. Scand J Infect Dis. 2009,41(6-7):410-5. doi: 10.1080/00365540902896079.

[5] Goldstein FW, Ly A, Kitzis MD. Comparison of Etest with agar dilution for testing the susceptibility of Pseudomonas aeruginosa and other multidrug-resistant bacteria to colistin. J Antimicrob Chemother. 2007,59(5):1039-40. doi: 10.1093/jac/dkm046.

[6] Bradford PA. Extended-spectrum beta-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev. 2001,14(4):933-51, doi: 10.1128/CMR.14.4.933-951.2001.

[7] David J, Hetem Miquel B, Ekkelenkamp.In Infectious Diseases. 4th ed. Jonathan Cohen, William G. Powderly and Steven M.2017, pp. 42-43.

[8] Fatemeh A, Emran A, Elnaz K, Mohammad J, G S, et al. The frequency of extended spectrum beta lactamase (ESBL) in Escherichia coli and Klebsiella pneumonia: a report from Mashhad, Iran. Journal of medical bacteriology.2012, 1(3): 12-19.

[9] Bonomo RA. β-Lactamases: A Focus on Current Challenges. Cold Spring Harb Perspect Med. 2017,7(1): a025239. doi: 10.1101/cshperspect.a025239.

[10] Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol Rev. 2005,18(4):657-86. doi: 10.1128/CMR.18.4.657-686.2005.

[11] Kaftandzhieva A, Kotevska V, Jankoska G, Trajkovska B K, Cekovska Z, et al. Extended-Spectrum Beta-Lactamase-Producing E. coli and Klebsiella Pneumoniae in Children at University Pediatric Clinic in Skopje. Macedonian Journal Medical Sciences.2009, 2 (1): 36-41.doi.org/10.3889/MJMS.1857-5773.2009.0030

[12] Chayakulkeeree M, Junsriwong P, Keerasuntonpong A, Tribuddharat C, and Thamlikitkul V. Epidemiology of extended-spectrum beta-lactamase producing gram negative bacilli at Siriraj hospital, Thailand 2003. Southeast Asian Journal of tropical medicine and public health.2005, 36(6): 1503-1509.

[13] Tumbarello M, Sali M, Trecarichi EM, et al. Bloodstream infections caused by extended-spectrum-beta-lactamase- producing Escherichia coli: risk factors for inadequate initial antimicrobial therapy. Antimicrob Agents Chemother. 2008;52(9):3244-3252. doi:10.1128/AAC.00063-08

DOI: https://doi.org/10.54393/pbmj.v5i4.258

[14] Tankhiwale SS, Jalgaonkar SV, Ahamad S, Hassani U. Evaluation of extended spectrum beta lactamase in urinary isolates. Indian J Med Res. 2004,120(6):553-6.
[15] Ramazanzadeh R. Etiologic Agents and Extended-Spectrum Beta-Lactamase Production in Urinary Tract Infections in Sanandaj, Iran. Eastern Journal of Medicine, 2010,15: 57-62.
[16] Pitout JD, Laupland KB. Extended-spectrum beta-lactamase-producing Enterobacteriaceae: an emerging public-health concern. Lancet Infect Dis. 2008,8(3):159–66. doi: 10.1016/S1473-3099(08)70041-0.
[17] Feizabadi MM, Delfani S, Raji N, Majnooni A, Aligholi M, et al. Distribution of bla(TEM), bla(SHV), bla(CTX-M) genes among clinical isolates of Klebsiella pneumoniae at Labbafinejad Hospital, Tehran, Iran. Microb Drug Resist. 2010,16(1):49–53. doi: 10.1089/mdr.2009.0096.
[18] Gashe F, Mulisa E, Mekonnen M, & Zeleke G. Antimicrobial ResistanceProfile of Different Clinical Isolates against Third-Generation Cephalosporins. Journal of pharmaceutics, 2018, 5070742. doi.org/10.1155/2018/5070742
[19] Hamidian M, Tajbakhsh M, Walther-Rasmussen J, Zali MR. The emergence of extended-spectrum beta-lactamases in clinical isolates of Salmonella enterica in Tehran, Iran. Jpn J Infect Dis. 2009,62(5):368–71.
[20] Tajbakhsh M, Garcia Migura L, Rahbar M, Svendsen CA, Mohammadzadeh M, et al. Antimicrobial-resistant Shigella infections from Iran: an overlooked problem? J Antimicrob Chemother. 2012;67(5):1128–33. doi: 10.1093/jac/dks023.
[21] Ensor VM, Jamal W, Rotimi VO, Evans JT, Hawkey PM. The predominance of CTX-M-15 extended-spectrum beta-lactamases in diverse Escherichia coli and Klebsiella pneumoniae from hospital and community patients in Kuwait. Int J Antimicrob Agents. 2009,33(5):487–9.doi:10.1016/j.ijantimicag.2008.10.011.
[22] Al-Agamy MH, Shibl AM, Tawfik AF. Prevalence and molecular characterization of extended-spectrum beta-lactamase-producing Klebsiella pneumoniae in Riyadh, Saudi Arabia. Ann Saudi Med. 2009,29(4):253–7.doi.org/10.4103/0256-4947.55306
[23] Yumuk Z, Afacan G, Nicolas-Chanoine MH, Sotto A, Lavigne JP. Turkey: a further country concerned by community-acquired Escherichia coli clone O25-ST131 producing CTX-M-15. J Antimicrob Chemother. 2008,62(2):284–8. doi: 10.1093/jac/dkn181.
[24] Albert MJ, Rotimi VO, Dhar R, Silpikurian S, Pacsa AS, et al. Diarrhoeagenic Escherichia coli are not a significant cause of diarrhea in hospitalized children in Kuwait. BMC Microbiol. 2009,9:62. doi: 10.1186/1471-2180-9-62.
[25] Zonneveld A, Al Dhaheri K, Mag T, Herpay M, Kolodziejek J, Nowotny N, et al. CTX-M-15-producing multidrug-resistant enteroaggregative Escherichia coli in the United Arab Emirates. Clin Microbiol Infect. 2006,12(6):582–5. doi: 10.1111/j.1469-0691.2006.01413.x.
[26] Vignoli R, Varela G, Mota MI, Cordeiro NF, Power P, et al. Enteropathogenic Escherichia coli strains carrying genes encoding the PER-2 and TEM-116 extended-spectrum beta-lactamases isolated from children with diarrhea in Uruguay. J Clin Microbiol. 2005,43(6):2940–3. doi: 10.1128/JCM.43.6.2940-2943.2005.
[27] Amaya E, Reyes D, Vilchez S, Paniagua M, Mollby R, et al. Antibiotic resistance patterns of intestinal Escherichia coli isolates from Nicaraguan children. J Med Microbiol. 2011,60(Pt 2):216–22. doi: 10.1099/jmm.0.020842-0.
[28] Bradford PA. Extended-spectrum beta-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev. 2001,14(4):933–51. doi: 10.1128/CMR.14.4.933-951.2001.
[29] Ingram PR, Inglis TJ, Vanzetti TR, Henderson BA, Harnett GB, et al. Comparison of methods for AmpC beta-lactamase detection in Enterobacteriaceae. J Med Microbiol. 2011,60(Pt 6):715–21. doi: 10.1099/jmm.0.029140-0.
[30] Benenson S, Temper V, Cohen MJ, Schwartz C, Hidalgo-Grass C, Block C. Imipenem disc for detection of KPC carbapenemase-producing Enterobacteriaceae in clinical practice. J Clin Microbiol. 2011,49(4):1617–20. doi: 10.1128/JCM.02179-10
[31] Blanco M, Blanco JE, Dahbi G, Mora A, Alonso MP, et al. Typing of intimin (eae) genes from enteropathogenic Escherichia coli (EPEC) isolated from children with diarrhea in Montevideo, Uruguay: identification of two novel intimin variants (muB and xiR/beta2B). J Med Microbiol. 2006,55(Pt 9):1165–74. doi: 10.1099/jmm.0.46518-0.
[32] Kozub-Witkowski E, Krause G, Frankel G, Kramer D, Appel B, Beutin L. Serotypes and virutypes of enteropathogenic and enterohaemorrhagic Escherichia coli strains from stool samples of children with diarrhoea in Germany. J Appl Microbiol. 2008,104(2):403–10. doi: 10.1111/j.1365-2672.2007.03545.x.
[33] Campos LC, Franzolin MR, Trabulsi LR. Diarrheagenic Escherichia coli categories among the traditional

DOI: https://doi.org/10.54393/pbmj.v5i4.258

enteropathogenic E. coli O serogroups—a review. Mem Inst Oswaldo Cruz. 2004,99(6):545–52. doi.org/10.1590/S0074-02762004000600001

[34] Nielsen JB, Albayati A, Jorgensen RL, Hansen KH, Lundgren B, et al. An abbreviated MLVA identifies Escherichia coli ST131 as the major extended-spectrum beta-lactamase-producing lineage in the Copenhagen area. Eur J Clin Microbiol Infect Dis. 2013,32(3):431–6. doi: 10.1007/s10096-012-1764-x.

[35] Naseer U, Olsson-Liljequist BE, Woodford N, Dhanji H, Canton R, et al. Multi-locus variable number of tandem repeat analysis for rapid and accurate typing of virulent multidrug resistant Escherichia coli clones. PLoS One. 2012,7(7). doi: 10.1371/journal.pone.0041232.

[36] Kanafani Z A, Dakdouki G K, El-Chammas, K I, Eid, S, et al. Bloodstreaminfections in febrile neutropenicpatients at a tertiary care center in Lebanon: a view of the past decade. Int. J. Infect. Dis. 2007,11: 450–453. doi: 10.1016/j.ijid.2006.12.008

[37] Zinner S H. Changing epidemiology of infections in patients with neutropenia and cancer: emphasis on gram-positive and resistant bacteria. Clin. Infect. Dis. 1999, 29: 490–494. doi: 10.1086/598620

[38] Ramphal R. Changes in the etiology of bacteremia in febrile neutropenic patients and the susceptibilities of the currently isolated pathogens. Clin. Infect. Dis. 2004,39: S25–S31. doi: 10.1086/383048

[39] Freifeld A G, Bow E J, Sepkowitz K A, Boeckh M J, Ito J I, et al.Infectious diseases society of america clinical practice guideline for the use of antimicrobial agents in neutropenic patients with cancer: 2010 update by the infectious diseases society of america. Clin. Infect. Dis. 2011,52: 427–431. doi: 10.1093/cid/ciq147

[40] Dettenkofer M, Ebner W, Bertz H, Babikir R, Finke J, et al. Surveillance of nosocomial infections in adult recipients of allogeneic and autologous bone marrow and peripheral blood stem-cell transplantation. Bone Marrow Transplant. 2003,31: 795–801. doi: 10.1038/sj.bmt.1703920