REFERENCES

1. **Ahmed, A., Li, J; Shiloach Y; Robbins J; Szu, S.** (2006). Safety and immunogenicity of *Escherichia coli* O157 O-specific polysaccharide conjugate vaccine in 2-5-year-old children. *J Infect Dis* 193 (4): 515–21.
2. **Akhan, S., F. Coskunkan, O. Tansel, and H. Vahaboglu. (**2001). Conjugative resistance to tazobactam plus piperacillin among extended-spectrum beta-lactamase-producing nosocomial *Klebsiella pneumoniae. Scand. J. Infect. Dis.* **33:**512–515
3. **Akinyemi KO., Alabi SA, Taiwo MA, Omonigbehin EA** (1997). Antimicrobial Susceptibility Pattern and Plasmid profiles of Pathogenic Bacteria isolated from Subjects with Urinary Tract Infections in Lagos, Nigeria. Niger. Qtr. *J. Hosp. Med.* 1: 7-11.
4. **Al-Agamy MHM., Ashour MSED, Wiegand I.** (2006). First description of CTX-M β-lactamase producing clinical *Escherichia coli* isolates from Egypt. *Int J Antimicrob Agents.* 2006; 27: 545-54.
5. **Ali, A, K.** (2012). Phenotypic detection and molecular characterization of extended-spectrum β-lactamases in Enterobacteria isolated from Khartoum hospital. *Sudan University of Science and Technology, Microbiology Dep*. October 2012.
6. **Alós, JI., Serrano, MG; Gómez-Garcés, JL;Perianes, J.** (2005) Antibiotic resistance of *Escherichia coli* from community-acquired urinary tract infections in relation to demographic and clinical data. *Clin Microbiol Infect.*
7. **Amaral S., L. Peixe, E Machado**. (2009). *Edicoes Universidade Fernando Pessoa*, 2009, 6, 259-263.
8. **Ambler, RP**. (1980). The structure of beta-lactamases. *Philos Trans R Soc Lond B Biol Sci*. 1980;289: 321-31 . 11:199-203.

9. **Andrea, E., Francesco, L; Gioconda, B; Mariagrazia, P; Gianlogi ,L ; Gianfranco, A., and Antoni, T** (2005); *Proteous mirabilis* blood stream infection : Risk factors and Treatment outcome related to expression of Extended-Spectrum beta lactamases. *Antimicrob Agents Chemother ;* 49(7):2598-2605.
10. **Andrea, J, L., and William, J. Brown**. (2005). Evaluation of Four Commercial Available Extended-Spectrum Beta-Lactamase Phenotypic confirmation Test. *J Clin Microbiol*;43(3): 1081-1085.
11. **Asinobi AO., Fatunde OJ, Brown BJ, Osinusi K, Fasina NA.** (2003). Urinary Tract Infection in Febrile Children with Sickle Cell Anemia inIbadan, Nigeria. Ann. *Trop. Paediatr.* 23(2): 129-134.
12. **Bach, S. J., T. A. McAllister, D.M. Veira, V.P.J. Gannon, and R.A. Holley**. (2002). Transmission and control of *Escherichia coli* O157:H7. *Canadian Journal of Animal Science* 82: 475–490.
13. **Bae IK., Woo G. J, Jeong SH, Park KO, Cho BK, Kim DM**. (2004). Prevalence of CTX-M-type extended-spectrum β-Lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* isolates in Korea. *Korean J Clin Microbiol* 2004;7:48-54.
14. **Ballabio, A., Ranier, JE. Chamberlain, JS, Zollo. M; Caskey, CT.** (1990). Screening for steroid sulfatase (STS) gene deletions by multiplex DNA amplification. *Human Genetics* 84 (6): 571–573.
15. **Baker, K.P., Baron, W.F., Henzel, W.J., and Spencer, S.A. 1998**. Molecular cloning and characterization of human and murine DNase II. Gene 215: 281–289.
16. **Bartlett, J.M., S. Stirling, D.** (2003). "A Short History of the Polymerase Chain Reaction". PCR Protocols. 226. pp. 3–6.

17. **Bauernfeind, A., Stemplinger, I. Jungwirth, R**. (1996).Characterization of beta - lactamase gene blaPER-2, which encodes an extended-spectrum class A ß-lactamase. *Antimicrob Agents Chemother* 1996; 40:616-620.
18. **Ben-Ami , R., Rodriguez-Bano, J. Arslan, H. Pitout, JDD. Quentin, C. Calbo ES, Azap OK, Arpin C, Pascual A, Livermore DM, Garau J, and Carmeli Y**. (2009). A multinational survey of risk factors for infection with extended-spectrum β-Lactamase–producing Enterobacteriaceae in nonhospitalized patients. *Clin. Infect. Dis*. 49: 682-690.
19. **Blattner, F. R., Plunkett, G. Bloch, C. A.; Perna, N. T.; Burland, V.; Riley, M.; Collado-vides, J. Glasner, J. D.** (1997). "The Complete Genome Sequence of *Escherichia coli* K-12". *Science* 277 (5331): 1453.
20. **Black, and J.G.** (1996). Microbiology. Principle and application .*Third edition* .Prentice Hall .Upper Saddle .*River, New Jersy*. Pp.436-443.
21. **Blomberg, B., Olsen BE, Hinderaker SG, Langeland N, Gasheka P, Jureen, R., Kvale G, Midtvedt T.** (2005**).** Antimicrobial resistance in urinary bacterial isolates from pregnant women in rural Tanzania: implications for republic health. *Scandinavian Journal of Infectious Diseases* 2005, 37(4):262-8.
22. **Bonnet, R. (**2004). Growing group of extended-spectrum ß-lactamases: the CTX-M enzymes. *Antimicrob. Agents Chemother.* 48:1–14.
23. **Bradford, P. A.** (2001). Extended-spectrum ß-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. *Clin Microbiol Rev* 14:933-51.
24. **Brüssow, H., Canchaya, C. Hardt, WD.** (2004). Phages and the evolution of bacterial pathogens: from genomic rearrangements to lysogenic conversion. *Microbiol. Mol. Biol. Rev*. 68 (3): 560–602.
25. **Burbige, KA., Retic AB, Colony A, Bauer SB, Lebowitz R.** (1984). UTI in boys. J. Urol. 132: 541-542.

26. **Bush, K., G. A. Jacoby, and A. A. Medeiros. (**1995). A functional classification scheme for b-lactamases and its correlation with molecular structure. *Antimicrob. Agents Chemother*. 39**:**1211–1233.
27. **Cao, V., Lambert, T. & Courvalin, P**. (2002). ColE1-like plasmid pIP843 of *Klebsiella pneumoniae* encoding extended-spectrum ß-lactamases CTX-M-17. *Antimicrob Agents Chemother* 46, 1212–1217.
28. **Carolina, F., Walter Z, Patrice F, Sasi D, Jacques S.** (2009). Surveillance of Extended-Spectrum-β-Lactamase-Producing Enterobacteriaceae in a Swiss Tertiary Care Hospital. *Swiss medically* 2009; 139(51–52): 747–751.
29. **Chalmers, R.M., H. Aird, F. J. Bolton.** (2000). "Waterborne *Escherichia coli O157*. Society for Applied Microbiology Symposium Series (29): 124S–132S.
30. **Chamberlain, JS., Gibbs, RA, Ranier, JE. Nguyen, PN. Caskey, CT** (1988). Deletion screening of the Duchenne muscular dystrophy locus via multiplex DNA amplification*". Nucleic Acids Research* 16 (23): 11141–11156.
31. **Cheesbrough, M.** (2000). District Laboratory Practice in Tropical Countries. *Cambridge United Press, U.K. part* 27: 105.
32. **Coque, TM. Baquero, F. Canton, R.** (2008). Increasing prevalence of ESBL producing *Enterobacteriaceae* in Europe. *Euro surveillance. Nov* 2008, 13 (47).
33. **Darnton, N. C., Turner, L. Rojevsky, S. Berg, H. C**. (1967). On torque and tumbling in swimming *Escherichia coli*. J *Bacteriol.* 189 (5): 1756–1764.
34. **De Boer, E. Heuvelink, AE.** (2000). Methods for the detection and isolation of Shiga toxin-producing *Escherichia coli. Symp Ser Soc Appl Microbiol* (29): 133S–143S.

35. **Dezful, Ahvaz, Jundishapur**. (2012). Distribution of TEM, SHV and CTX-M Genes among ESBL-producing Enterobacteriaceae isolates in Iran. *African Journal of Microbiology Research Vol.* 6(26), pp. 5433-5439, 12 July, 2012
36. **Ehrlich, G., Hu, F. Shen, K. Stoodley, P. Post, J**. (2005). Bacterial plurality as a general mechanism driving persistence in chronic infections. *Clin Orthop Relat Res* (437): 20–4.
37. **Emery, C, L., and Weymouth, L. A.** (1997). Detection and clinical significance of extended-spectrum β-lactamases in a tertiary-care medical center J. *Clin. Microbiol.* 35:2061-206.
38. **Evans, Jr., Doyle, J. Dolores, G. Evans**. (2007). *Escherichia Coli*". *Medical Microbiology, 4th edition*. The University of Texas Medical Branch at Galveston. Archived from the original on 2007-11-02. Retrieved 2007-12-02.
39. **Eslam, A. A**. (2010). Molecular characterization of extended-spectrum β-lactamases in *Salmonellae spp*. isolated from clinical specimens. Khartoum, *Sudan University of Science and Technology, Microbiology Dep*. 2010.
40. **Feng, P., Weagant, S. Grant, M.** (2002). Enumeration of *Escherichia coli* and the Coliform Bacteria". *Bacteriological Analytical Manual (8th ed.).* FDA/Center for Food Safety & Applied Nutrition. Retrieved 2007-01-25.
41. **Foster, TJ.** (1983). Plasmid-determined resistance to antimicrobial drug and toxic metal ions in bacteria. *Microbiology Rev,* 47; 361, 1983.
42. **Fotadar, U., Zaveloff, P. Terracio, L.** (2005). Growth of *Escherichia coli* at elevated temperatures. *J. Basic Microbiol.* 45 (5): 403–4.
43. **Garrec, H., L. Drieux-Rouzet, JL. Golmard, V. Jarlier, J. Robert**. (2011). *J Clin Microbiol*, **2011**, 49, 3, 1048-1057.

44. **Girard, M., Steele, D. Chaignat, C. Kieny, M**. (2006). A review of vaccine research and development: human enteric infections. *Vaccine* 24 (15): 2732–50.
45. **Girard, M., Steele, D. Chaignat, C. Kieny, M.** (2006). A review of vaccine research and development: human enteric infections. *Vaccine* 24 (15): 2732–50.
46. **Ghatole, M. Manthalkar, P. Kandle, S. Yemul, V. Jahagirdar, V.** (2004). Correlation of extended spectrum b-lactamases production with Cephalosporin resistance in gram negative bacilli. Indian *J Pathol Microbial* 2004; 47(1):82-84.
47. **Gonzalez, CM., Schaeffer, AJ.** (1999). Treatment of urinary tract infection: what's old, what's new, and what works. *World J Urol* 1999, 17:372-382.
48. **Gupta, K., Scholes, D. Stamm, WE**. (1999). Increasing prevalence of antimicrobial resistance among uropathogens causing acute uncomplicated cystitis in women. *JAMA* 281:736-738.
49. **Haider, G., Zehra, N. Munir, AA. Haider, A.** (2010). Risk factors of urinary tract infection in pregnancy. J *Pak Med Assoc* 2010, 60(3):213-6.
50. **Hamdan, Z., Hamdan, Abdel Haliem M Ziad, Salah K Ali, Ishag Adam, (2011).** Annals of Clinical Microbiology and Antimicrobials 2011, 10:2**,** http://www.ann-clinmicrob.com/content/10/1/2**.**
51. **Humeniuk, C.** (2002). Beta-lactamases of *Kluyvera ascorbata*, probable progenitors of some plasmid-encoded CTX-M types. *Antimicrob Agents Chemother* 46:3045-9. Madison Department of Bacteriology. *Retrieved* 2007-11-30.
52. **Ibeawuchi**, **R., Mbata, TI.** (2002). Rational and Irrational Use of Antibiotics. Afr. Health. 24 (2): 16-18.
53. **Ingledew, WJ., Poole, RK.** (1984). The respiratory chains of *Escherichia coli. Microbiol. Rev*. 48 (3): 222–71.

54. **Jacoby, AG**. (2005) Munoz-Price LS. Mechanisms of disease the new β-lactamase. *N Engl J Med.* 2005; 325: 380-91.

55. **Johnson, JR., Russo, TA**. (2002). Extra intestinal pathogenic *Escherichia coli* : the other bad *E . coli. J Lab Clin Med* 2002; 139 :155-62.

56. **Justice, S., Hunstad, D. Seed, P. Hultgren, S.** (2006). Filamentation by *Escherichia coli* subverts innate defenses during urinary tract infection. *Proc Natl Acad Sci U S A* 103 (52): 19884–9.

57. **Kahlmeter, G**. (2003). An international survey of the antimicrobial susceptibility of pathogens from uncomplicated urinary tract infections: the ECO-SENS project. *J Antimirobial Chemother* 51: 69-76.

58. **Kiratisin, P., Apisarnthanarak, A. Laesripa, C. Saifon, P.** (2008). Molecular characterization and epidemiology of extended-spectrum ß-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* isolates causing health care-associated infection in Thailand, where the CTX-M family is endemic. *Antimicrob Agents Chemother* .2008, 52:2818-2824.

59. **Knothe, H., Shah, P. Kremery, V.** (1983). Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of *Klebsiella pneumoniae* and *Serratia marcescens* Infection. 1983; 11: 315-7

60. **Kola, A., Holst, M. Chaberny, IF. Ziesing, S. Suerbaum, S. Gastmeier P.** (2007). Surveillance of extended-spectrum beta-lactamase-producing bacteria and routine use of contact isolation: experience from a three year period. *J Hosp Infect*. 2007;66:46–51.

61. **Kubitschek, HE**. (1990). Cell volume increase in *Escherichia coli* after shifts to richer media. *J. Bacteriol.* 172 (1): 94–101.

62. **Livermore, D. M. (**1995). b-Lactamases in laboratory and clinical resistance .*Clin. Microbiol. Rev.* 8:557–584.

63. **Luzazaro, F., Mezzatesta, M. Mugnaioli, C. Perlli, M. Stetani, Amicosante, G. Rossolini, G. M and Toriiolo, A.** (2006). Trend in

production of extended spectrum beta lactamase among *Enterobacteria* of medical interest: Report of the second Italian nationwide survey. *J. Clinical Microbiology* (44) : 1659-1664.

64. **Madigan, MT., Martinko, JM.** (2006). Brock Biology of microorganisms (11th ed.). Pearson. ISBN 0-13-196893-9.
65. **Madigan, MT., Martinko, J. and Parker, J**. (2004). *Brock Biology of Microorganisms 10th ed* Lippincott Williams & Wilkins. ISBN 0-13-066271-2.
66. **Mathai, E., Thomas, RJ. Chandy, S. Mathai, M. Bergstrom, S.** (2004). Antimicrobials for the treatment of urinary tract infection in pregnancy: practices insouthern India. *Pharmacoepidemiol Drug Saf* 2004, 13(9):645-52.
67. **Mathur, P., Kapil, A. Das, B. Dhawan, B**. (2002). Prevalence of ESBL producing gram negative bacteria in a tertiary care hospital. *Indian J Med Res* 2002; 115 :153-7.
68. **Mbata, TI**. (2007). Prevalence and Antibiogram of U T Is Among Prisons Inmates in Nigeria. Int. *J. Microbiol*. 3 (2).
69. **Migula, W**. (1985). Bacteriaceae (Stabchenbacterien). In: A. ENGLER and K. PRANTL (eds): Die Naturlichen Pfanzenfamilien, W. Engelmann, Leipzig, Teil I, Abteilung Ia, 11985, pp. 20–30.
70. **Moubareck, C., Z. Daoud, N. I. Hakime, M. Hamze, N. Mangeney, H. Matta, J. E. Mokhbat, R. Rohban, D. K. Sarkis, and F. Doucet-Populaire.** 2005. Countrywide spread of community- and hospital acquired extended-spectrum beta-lactamase (CTX-M-15)-producing *Enterobacteriaceae* in Lebanon. *J. Clin. Microbiol*. 43**:**3309-3313
71. **Moland, E. S., Black, J. A. Hossain, A. Hanson, N. Thomson, D. K. S. and Pottumarthy, S. (**2003). Discovery of CTX-M-like extended-spectrum

_-lactamases in *Escherichia coli* isolates from five U.S. *states. Antimicrob. Agents Chemother*. 47:2382–2383.

72. **Murray, PR., Baron, EJ. Jorgensen, JH. Landry, ML. Michael, A. Pfaller, MA. (**2007). *Manual of Clinical microbiology. 9th ed.* Washington: American Society for Microbiology.
73. **Naas, T. and Nordmann, P. (**1999). OXA-type b-lactamases. Curr. Pharm.Des. 5**:**865–879.
74. **Nordmann, P.** (1998). Trends in b-lactam resistance among *Enterobacteriaceae*. Clin. Infect. Dis. 27(Suppl. 1)**:**S100–S106.
75. **Paterson, D**. **L**.(2005). Extended-spectrum ß-lactamases: a clinical update. *Clin Microbiol Rev* 18:657-86.
76. **Paterson, DL., Hujer, KM. Hujer, AM.** (2003). Extended-spectrum b-lactamases in *Klebsiella pneumoniae* bloodstream isolates from seven countries: dominance and widespread prevalence of SHV- and CTX-M-type b-lactamases. *Antimicrob Agents Chemother.* 2003; 47:3554-60.
77. **Paton, JC. Paton, AW.** (1998). "Pathogenesis and diagnosis of Shiga toxin-producing *Escherichia coli* infections". *Clin. Microbiol. Rev.* 11 (3): 450–79. PMID 9665978.PMC 88891.
78. **Picard, B. Garcia, JS. Gouriou, S. Duriez, P. Brahimi, N. Bingen, E. (**1999). The link between phylogeny and virulence in *Escherichia coli* extra intestinal infection. *Infect Immun* 1999; 67 :546.
79. **Pitout, J. D. D., D. B. Gregson, D. L. Church, S. Elsayed, and K. B. Laupland.** (2005). Community-wide outbreaks of clonally related CTX-M-14 β-lactamase-producing Escherichia coli strains in the Calgary health region. *J. Clin. Microbiol.* 43:2844-2849.
80. **Philippon, A., Arlet, B. Jacoby, GA**. (2002). Plasmid-determined AmpC-type β-lactamases *Antimicrob Agents Chemother.*. 2002; 46 .

81. **Podschun, R. and Ullmann U**. (1998). Extended –spectrum beta-Lactamases: a clinical update . *Clinical Microbiol Rev* ; 18:657-86.Poole K. Resistance to *β*-lactam antibiotics. *CMLS, Cell Mol Life Sci*. 2004; 61: 2200- 23.
82. **Poirl, L., Nass, T, Guibert, M. Chaibi, B. Labia, R. Nordmann, P**. (1996). Molecular and biochemical aracterization of VEB-1, anovel class A extended-spectrum beta-lactamase encoded by an *Escherichia coli* integron gene. Antimicrob Agents Chemother 1999; 43:573-581.
83. **Ravi, S., Giriabur, Namaratha, W. and Kirishna, BVS**. (2012). Comparison of disc diffusion methods for detection of ESBLs producing Enterobacteriaceae. PES institute of medical science kuppm. September, 2012 .IPS 197.252.1.72.
84. **Romero, L., L. Lopez, J. Rodriguez-Bano, J. Ramon Hernandez, L. Martinez-Martinez, and A. Pascual**. (2005). Long-term study of the frequency of *Escherichia coli* and *Klebsiella pneumoniae* isolates producing extended-spectrum beta-lactamases. *Clin. Microbiol. Infect.* 11:625-631.
85. **Ruppé, E.** (2010). Épidémiologie des ß-lactamases à spectre élargi: l'avènement des CTX-M. Antibiotiques 2010, 12:3-16.
86. **Salyers, AA. Gupta, A. Wang, Y**. (2004). Human intestinal bacteria as reservoirs for antibiotic resistance genes. *Trends Microbiol.* 12 (9): 412–6. doi:10.1016/j.tim.2004.07.004.
87. **Saiki, R., Scharf, S. Faloona, F. Mullis, K. Horn, G. Erlich, H. Arnheim, N.** (1985). Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* 230 (4732): 1350–1354.
88. **Sekar, B. Shwetha, R. Arunagiri, K. Menaka, K. Lalitha, P. Aparna V.** (2006). Detection and characterization of bla CTX-M gene by PCR-RFLP analysis among third generation cephalosporin resistant gram negative

isolates. XXX National congress of Indian Association of Medical Microbiologists. *Microcon*: 2006. p. 27.

89. **Silva, J., Aguiler, C. Ayla, G. TLA-1.** (2000). A new plasmid-mediated extended-spectrum beta-lactamase from *Escherichia coli*. *Antimicrob Agents Chemother* 2000; 44:997- 1003.
90. **Song, W., Kim, JS. Kim, MN. Kim, EC. Park, YJ. Yong, D**. (2002). Occurrence and genotypic distributions of plasmid-mediated AmpC beta-Lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in Korea. *Korean J Lab Med* 2002;22:410-6.
91. **Steckelberg, JM. (**2009). *Escherichia coli.* Centers for Disease Control and Prevention. Accessed May 5, 2009. (expert opinion). Mayo *Clinic, Rochester,* Minn. May 12, 2009.16.
92. **Strom, BL**. (1987). Sexually activity, contraceptive use, and other risk factor for symptomatic an a symptomatic bacteriuria. A case-control study. *Annals intmed*; 107:816-23.
93. **Tauschek, M., Gorrell, R. Robins-Browne, RM. (**2001). Identification of a protein secretory pathway for the secretion of heat-labile enterotoxin by an enterotoxigenic strain of *Escherichia coli*. PNAS 99: 7066–71.
94. **Tasli, H. Bahar, IH**. (2005). Molecular characterization of TEM and SHV derived ESBL In hospital based Enterobacteriaceae in Turkey. *Jpn. J. Infect. Dis*. 58:162-167.
95. **Tenover, FC., PM. Raney, PP. Williams, JK. Rasheed, JW. Biddle, A. Oliver, S. K. Fridkin, L. Jevit**t. (2003). JE McGowan. *J Clin Microbiol*, 2003, 41, 7, 3142-3146.
96. **Todar, K**.(2007). Pathogenic *E. coli*, Online Textbook of Bacteriology. University of Wisconsin–Madison Department of Bacteriology. *Retrieved* 2007-11-30.

97. **Thomas, R. DeGregori.** (2007). CGFI: Maddening Media Misinformation on Biotech and Industrial Agriculture. *Retrieved* 2007-12-08.
98. **Tzouvelekis, L. S., E. Tzelepi, P. T. Tassios, and N. J. Legakis. (**2000). CTXM-type b-lactamases: an emerging group of extended-spectrum enzymes. Int. J. *Antimicrob. Agents* 14:137–142.
99. **Wayne, PA.** (1998). National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing. NCCLS, Supplement Tables M100-S8 to Methods for dilution antimicrobial susceptibility test for bacteria that grew aerobically fourth edition: approved standard.
100. **Wayne, PA**. (2006). Clinical and Laboratory Standards Institute: *Ninth edition Document M2-A9* Clinical and Laboratory Standards Institute, Performance standards for antimicrobial disk susceptibility tests. Approved standard; 2006.
101. **Wayne, PA.** (2002). National Committee of Clinical Laboratory Standards. Performance standard for antimicrobial disk susceptibility testing; Twelfth Informational Supplement NCCLS Documents M100-S12. NCCLS: (Pa): Wayne (PA): 2002.
102. **Woodford, N. Ward, E. Kaufmann, ME.** (2006). Molecular characterization of *Escherichia coli* isolates producing CTX-M-15 extended-spectrum β-lactamase (ESBL) in the United Kingdom (PDF). *Health Protection Agency*. Retrieved 2006-11-19.
103. **Winokur, PL., Canton, R. Casellas, JM**. (2001). Variations in the prevalence of strains expressing an extended-spectrum b-Lactamase phenotype and characterization of isolates from Europe, the Americas, and the Western Pacific Region. *Clin Infe Dis*. 2001;32:94–103.

104. **Zong, Z. Partridge, S. Thomas, L. Iredell, JR**. (2008). Dominance of the *bla*CTX-M within an Australian extended-spectrum β-lactamase gene pool. *Antimicrob Agents Chemother*. 2008; 52: 4198-202.

APPENDIX 1

THE COLOR PLATES

Figure (8) Colonial morphology of isolated *E.coli* on blood agar

Figure (9) Colonial morphology of isolated *E.coli* on Mac Conkey agar

Figure (10) Gram stain of isolated *E.coli*

Figure (11) Indole test

Figure (12) Citrate test

Figure (13) Catalase test

Figure (14) The biochemical tests of *E.coli* isolate

APPENDIX II

PREPARATION OF REAGENTS AND CULTURE MEDIA

1. Blood agar base

Blood agar base is recommended as base to which blood may be added for use in the isolation and cultivation of fastidious pathogenic microorganisms.

Compositions

Ingredients	**Gms/L**
Beef heart, infusion (beef extract)……	5000
Tryptose ……..10	
Sodium chloride …….5	
Final pH ……...…7.3	

Directions

Suspend 40 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterile by Autoclave at 15 ibs pressure (121℃) for 15 min. Cool to 50℃ and aseptically add 5% v/v sterile defibrinated blood. Mix well and pour into sterile petridishes.

2. Crystal violet Grams stain

To make 1 liter

Crystal violet…… 20 g

Ammonium oxalate ……9 g

Ethanol or methanol , absolute ……95ml

Distilled water ……to 1 litter

3. Weight the crystal violet on a piece of clean paper. Transferred to a brown bottle pre marked to hold one litter
4. Add the absolute ethanol or methanol and mix until the dye is completely dissolved.
5. Weight the ammonium oxalate and dissolve in about 200 ml of distilled water. Add the stain, make up to one litter with distilled water and mixed well.
6. Label the bottle and store it at room temperature. The stain is stable for several months.

3. Kliger Iron Agar (KIA)

KIA reactions are based on the fermentation of lactose and glucose (dextrose) and the production of hydrogen sulphide.

Compositions

Ingredients	**Gms/L**
Peptic digest of animal tissue……	15
Yeast extract……	3
Beef extract……..…	3
Peptose peptone ……...…	5
Dextrose …….	1
Lactose ……..……	10
Ferrous sulphate ……...	0.20
Sodium chloride ……..…..5	
Sodium thiosulphate…….. .0.3	
Phenol red…… …	0.042
Agar……..	15
Final pH(at 25℃)……	7.4

Directions

Suspend 57.5 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterile by Autoclave at 15 ibs pressure (121℃) for 15 min. mix and pour. set as slope with butt.

1. Lugol's iodine solution

To make one litter

Potassium iodine solution ……20 g

Iodine …….. 10 g

Distilled water ……to 1 litter

1. Weight the potassium iodine , and transfer to brown bottle pre marked to hold 1 litter .
2. Add about quarter of the volume of water, and mix until the potassium iodine solution is completely dissolved .
3. Weight the iodine, and add to potassium iodide solution. Mix until the iodine is dissolved .
4. Make up to 1 litter distilled water, mix well. Label the bottle and marked toxic. Store at dark place

5. Mac Conkey Agar medium

Mac Conkey Agar medium is a differential medium to distinguish between bacteria by neutral red indicator which changes colour when acid is produced following fermentation of lactose sugar.

Composition

Ingredients	**Gms/L**
Peptic digest of animal tissue……..…	17
Protease peptone…….….	3
Lactose…….....	10
Bile salts ……	1.5
Sodium chloride ……..5	
Neutral red……	.03
Agar……..	15
Final pH(at 25℃)……	7.2

Directions

Suspend 51.53 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterile by Autoclave at 15 ibs pressure (121℃) for 15 min. mix and pour.

6. McFarland Standard Turbidity tube 0.5 :

Ingredients

Conc. Sulphuric acid ……..……1 ml

Dehydrated barium chloride ……..……..0.5g

Distilled water ……..……..……99 ml

Prepare 1% V/V of sulphuric acid solution by adding 1 ml of concentrated sulphuric acid to 99 ml of DW and mix. Prepare 1% w/v solution of barium chloride by dissolve 0.5g of dehydrated barium chloride in 50 ml of distilled water. Add 0.6 ml of sulphuric acid then mix well.

7. Muller Hinton agar

Muller Hinton agar is used for testing susceptibility of common and rabidly growing bacteria using antimicrobial disc, it manufactured to contain low level of thymine, thymidine, calcium and magnesium.

Compositions

Ingredients	**Gms/L**
Casein acid hydrolysate ……	17
Beef heart infusion ……. ……	2
Starch soluble ……	1.5
Agar……	17
Final pH(at 25℃)……....	7.3

Directions

Suspend 38 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterile by Autoclave at 15 ibs pressure (121℃) for 15 min. mix and pour.

8.Nutrient agar

Nutrient agar is used for cultivation of less fastidious organisms, can be enriched with blood or other biological fluids.

Compositions

Ingredients	**Gms/L**
Peptone …….……	10
Beef extract ……. ……...	10
Sodium chloride ……5	
Yeast xtract……1.5	
Agar……15	
Final pH(at 25℃)……...	7.3

Directions

Suspend 28 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterile by Autoclave at 15 ibs pressure (121℃) for 15 min. mix and pour.

1. Oxidase Reagent

Prepare fresh before use.

To make 10 ml:

Tetramethyle-*p*-phenylenediamine dihydrochloride …….0.1 g

Distilled water ……10ml

Dissolve the chemical in water . The reagent is not stable .

2. Peptone water

Used for culturing organisms to proceed indole test in the presence of Kovac's or Ehrlich's reagent that reacts with the indole to produce a red coloured compound.

Compositions

Ingredients	**Gms/L**
Peptic digest of animal tissue……	10
Sodium chloride ……..5	
Final pH(at 25℃)……..7.2	

Directions

Suspend 15 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterile by Autoclave at 15 ibs pressure (121℃) for 15 min. mix and pour.

3. Simmons citrate Agar

This test is used to assist in the identification of enterobacteria. The test is based on the ability of an organism to use citrate as its only source of carbon.

Compositions

Ingredients	**Gms/L**
Magnesium sulphate ……	0.20
Ammonium dihydrogen phosphate …….	1
Dipotassium phosphate ……...	1
Sodium citrate ……..2	
Sodium chloride …….5	
Bromothymol blue …….0.08	
Agar……..	15
Final pH(at 25℃)……	6.8

Directions

Suspend 24.28 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterile by Autoclave at 15 ibs pressure (121℃) for 15 min. mix and pour. set as slope .

4. Urea Agar Base (Christensen)

Testing for Urease enzyme activity is important in differentiating enterobacteria. Especially for *proteus spp*.

Compositions

Ingredients	**Gms/L**
Peptic digest of animal tissue……	1
Dextrose …….	1
Disodium phosphate ……...	1.20
Monopotassium phosphate …….	0.80
Sodium chloride ……..…..….	5
Phenol red…… ..0.012	
Agar……..15	
Final pH(at 25℃)……	6.8

Directions

Suspend 24 grams in 950 ml distilled water. Heat to boiling to dissolve the medium completely. Sterile by Autoclave at 10 ibs pressure (115℃) for 20 min. Cool to 50℃ and a aseptically add 50 ml of sterile 40% of urea solution (FD048) and mix.

APPENDIX III

INSTRUMENTS

Autoclave	Dixon,s	USA
Incubator	GALLENKAMD	UK
Hot air oven	leader engineering winnes Cheshire	UK
Water bath	Cout Zarf DIN Co,	Germany
Dry cabinet	leader engineering winnes Cheshire	UK
Microscope	Olympus optical. LTD	UK
Sensitive balance	ERN model AIS 120-4	Germany
Refrigerator	Starlet Europe	Europe
Safety cabinet	Daihan lab tech. LTD	UK

APPENDIX IV

QUESTIONNAIRE MODEL

Date……

Patient name ……

Specimen number ……

Age……

Gender Male ◯

Female ◯

Usage of antibiotic within last 2 weeks YES ◯

NO ◯

Types of antibiotic

……

……..

APPENDIX V

Table 1. The results of ESBLs by screening tests by using cefotaxime and ceftazidime discs

NO	CAZ	RESULT	CTX	RESULT
1	27	_	36	_
2	25	_	27	_
3	28	_	32	_
4	10	+	8	+
5	22	_	28	_
6	8	+	8	+
7	10	+	7	+
8	8	+	7	+
9	0	+	0	+
10	11	+	0	+
11	0	+	0	+
12	14	+	10	+
13	26	_	30	_
14	30	_	32	_
15	17	+	20	+
16	14	+	26	+
17	22	_	20	_
18	24	_	30	_
19	22	_	27	_
20	12	+	0	+
21	9	+	0	+
22	25	_	28	_
23	20	+	20	+
24	28	_	29	_
25	13	+	8	+

26	0	+	0	+
27	24	_	22	+
28	14	+	8	+
29	0	+	0	+
30	26	_	30	_
31	8	+	0	+
32	23	_	13	+
33	8	+	0	+
34	22	_	0	+
35	32	_	30	_
36	25	_	30	_
37	29	_	30	_
38	0	+	0	+
39	22	_	30	_
40	30	_	33	_
41	13	+	0	+
42	28	_	34	_
43	30	_	35	_
44	30	_	36	_
45	22	_	36	_
46	27	_	30	_
47	20	+	30	_
48	30	_	38	_
49	26	_	38	_
50	26	_	38	_
51	21	+	13	+
52	22	_	37	_
53	20	+	9	+
54	29	_	38	_

55	18	+	9	+
56	30	_	37	_
57	29	_	36	_
58	30	_	36	_
59	30	_	30	_
60	25	_	13	+
61	28	_	26	+
62	0	+	0	+
63	22	_	24	+
64	28	_	30	_
65	16	+	12	+
66	13	+	14	+
67	30	_	36	_
68	8	+	0	+
69	22	_	23	+
70	28	_	34	_
71	30	_	35	_
72	30	_	34	_
73	18	+	8	+
74	27	_	27	_
75	27	_	28	_
76	30	_	33	_
77	15	+	8	+
78	26	_	29	_
79	16	+	0	+
80	13	+	0	+
81	8	+	0	+
82	23	_	9	+
83	25	_	30	_

84	10	+	0	+
85	28	_	32	-
86	18	+	10	+
87	28	_	28	_
89	25	_	30	_
90	0	+	10	+
91	30	_	30	_
92	16	+	21	+
93	30	_	35	_
94	30	_	36	_
95	22	_	36	_
96	27	_	30	_
97	20	+	30	+
98	30	_	38	_
99	26	_	38	_
100	26	_	38	_
101	21	+	13	+
102	22	_	37	_
103	20	+	9	+
104	29	_	38	_
105	18	+	9	+
106	30	_	37	_
107	29	_	36	_
108	30	_	36	_
109	30	_	30	_
110	25	_	13	+
111	28	_	30	_
112	0	+	0	+
113	22	_	24	+

114	28	_	30	_
115	16	+	12	+
116	13	+	14	+
117	30	_	36	_
118	8	+	0	+
119	22	_	23	+
120	28	_	34	_
121	30	_	35	_
122	30	_	34	_
123	18	+	8	+
124	27	_	27	_
125	27	_	28	_
126	30	_	33	_
127	15	+	8	+
128	26	_	29	_
129	16	+	0	+
130	13	+	0	+
131	8	+	0	+
132	23	_	29	_
133	25	_	30	_

Table 2. Result of phenotypic detection of ESBLs by Phenotypic confirmatory tests (PCT)

NO	CAZ	CAZ+ CA	DIFFR ENCE	RESUL T	CTX	CTX+C A	DIFFRE NCE	RES ULT
1	10	25	15	+	8	20	12	+
2	8	18	10	+	8	20	12	+
3	10	26	16	+	7	12	5	+
4	8	25	17	+	7	31	6	+
5	0	23	23	+	0	18	18	+
6	11	20	9	+	0	20	20	+
7	0	24	24	+	0	22	22	+
8	10	30	20	+	10	15	5	+
9	12	28	16	+	0	15	15	+
10	9	27	18	+	0	22	22	+
11	13	26	13	+	8	16	8	+
12	10	23	13	+	0	19	19	+
13	0	24	24	+	0	16	16	+
14	14	30	16	+	8	20	12	+
15	0	25	25	+	0	20	20	+
16	26	28	2	_	0	22	22	+
17	13	30	17	+	13	26	13	+
18	8	27	19	+	0	20	20	+
19	0	20	20	+	0	18	18	+
20	0	23	23	+	0	20	20	+
21	13	22	9	+	0	15	15	+
22	21	25	4	_	13	23	10	+
23	20	23	3	_	9	20	11	+
24	18	25	7	+	9	22	13	+
25	25	30	5	+	13	18	5	+

26	21	25	4	_	27	32	5	+
27	16	19	3	_	0	10	10	+
28	26	26	0	_	12	22	10	+
29	13	25	12	+	14	20	6	+
30	8	26	18	+	0	15	15	+
31	18	25	7	+	8	20	12	+
32	15	28	13	+	8	28	20	`+
33	16	26	10	+	0	16	16	+
34	13	20	7	+	0	16	16	+
35	8	23	15	+	0	8	8	+
36	21	25	4	_	9	15	6	+
37	10	25	15	+	0	11	11	+
38	10	30	20	+	12	32	20	+
39	18	25	7	+	10	15	5	+
40	17	25	8	+	7	20	13	+
41	20	25	5	+	10	15	5	+
42	18	28	10	+	0	20	20	+
43	14	24	10	+	0	14	14	+
44	0	24	24	+	0	20	20	+
45	16	24	8	+	0	16	16	+
46	18	25	7	+	8	8	0	_
47	10	23	13	+	0	19	19	+
48	10	24	14	+	14	18	2	_
49	0	0	0	_	8	8	0	_
50	0	0	0	_	0	0	0	_
51	6	6	0	_	0	0	0	_
52	0	0	0	_	0	0	0	_

Table 3. Cut-off points of initial screening and confirmatory tests for ESBL production(Wayne ., 2002**)**

Initial screening test	**Phenotypic confirmatory test**	A ≥ 5-mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone may indicate ESBL production
Cefotaxime (30 µg) zone	< 27 mm	
Ceftazidime (30 µg) zone	< 22 mm	

Table 4. Antibiotic discs used for ESBLs confirmatory tests (NCCLS confirmatory test)

antibiotics	Potency	Symbol	Source
Ceftazidime	30µg	CAZ	Mast, UK
Ceftazidime/clavulanate	30 µg/10 µg)	CAC	Mast, UK
Cefotaxime	30µg	CTX-M	Mast, UK
Cefotaxime/clavulanate	30 µg/10 µg)	CEC	Mast, UK

Table 5. Presence/absence of SHV, CTX-M and TEM genes in samples resistant/susceptible to the third generation Cephalosporins by PCR

Total samples (N= 133)	SHV	CTX_M	TEM	TEM, SHV	TEM, CTX-M	SHV, CTX-M	TEM, SHV, CTX-M
ESBL positive (N=46)	3	28	9	0	2	0	0
ESBL negative(87) (((N=87)	0	0	4	0	0	0	0