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Antibiotic Resistance Profile of *Escherichia coli* and *Klebsiella pneumoniae* Isolated from Patients with Cystitis

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ABSTRACT

Urinary tract infections seem most important and prevalent hospital and community associated infections. It can be unimicrobial or polymicrobial with most prevalent microbes like Escherichia coli. And Klebsiella pneumoniae. It is hard to cured may be due to fluctuated antibiotic resistance mediated by arrays of mechanisms like modifying enzyme and efflux pumps. The study included 50 isolates of each E. coli. and K. pneumoniae confirmed by species specific genes, uidA and tryB. Antibiotic susceptibility for 25 antimicrobial agents were assessed. The results revealed that antibiotic susceptibility of E. coli, for penicillin and cephalosporin the results revealed that it is fully resist to amoxicillin, ceftazidime and cefotaxime while express moderate resistance to ceftriaxone (54%), cefixime (52%) and Cefoxitin (42%). The isolates express high sensitivity for piperacillin (74%). Also the susceptibility results to monobactam and carbapenem revealed that, (20%, 0.00% and 6%) were resistant to Aztreonam, imipenem and meropenem respectively while high sensitivity were documented (96%, 80% and 56%) for imipenem and meropenem, Aztreonam respectively. For quinolones, the isolates show low resistance (26%, 14% and 12%) for ciprofloxacin , ofloxacine and levofloxacin respectively. At same time they are express high sensitivity rate (82% and 80%) to ofloxacin and levofloxacin respectively. Susceptibility of E. coli to aminoglycosides revealed that moderate resistance to kanamycin , streptomycin and tobramycin (42%, 36% and 20% respectively). High sensitivity were stated (94%, 62%, 50%, 50%) to netilmicine, gentamycin amikacin and tobramycin respectively. Also the isolates show high sensitivity to azithromycin (90%), trimethoprim (64%), doxycycline (62%) and nalidixic acid (62%).The results of K. pneumoniae susceptibility revealed that high resistance to penicillins and cephalosporins (ceftazidime 98%, ceftriaxone 92%, cefotaxime 92%, amoxicillin 92%, cefixime 84% and cefepime 80%). It is only sensitive to piperacillin (66%). Additionally the isolates express high sensitivity to meropenem (86%), and imipenem (76%) while moderate resistance to Aztreonam (58%). Quinolones susceptibility results reveal that, they are highly sensitive to ofloxacin (90%) and moderate sensitivity to levofloxacin (58%) and ciprofloxacin (46%). High level of sensitivity were conveyed to netlimicin (92%), amikacin (82%) and moderate sensitivity to gentamycin (52%) while high resistance was for streptomycin (78%). They show high sensitivity to azithromycin (76%) and nalidixic acid (68%) while express high resistance to trimethoprim (76%) and nitrofurantion (58%). The current study conclude that, the most suitable drugs for UTIs-associated E. coli/K. pneumoniae were piperacillin, netilmicine, amikacin, imipenem, meropenem, levofloxacin. There is an increased emergence of resistance to cephalosporins along nitrofurantion and trimethoprime.

Introduction

Urinary tract infections (UTIs) are one of the most common bacterial infections and are causedby both Gram-negative and Gram-positive bacterium [1]. It account for 32% of major healthcare-associated infections [2]. *Escherichia coli (E. coli)* is reported as the most common pathogen of UTIs, followed by the second most common, *Klebsiella pneumoniae(K. pneumoniae)* [3]. UTI is a common painful human illness that, unfortunately not responsive to commonly used antibiotics in the current practice [4]. Drug resistance among Gram-negative pathogens is a risk factor for inappropriate empiric treatment which in turn increases the risk for mortality[5]. Antimicrobial resistance is increasing among uropathogens and the production of β-lactamases is a major resistance mechanism. Extended-spectrum beta-lactamase produced by *E. coli* and *K. pneumoniae* reduces the number of therapeutic options for the infection caused by these pathogens[6]. ESBL producer are resistant to penicillins, cephalosporins, and monobactam. The ESBL producers can also develop co-resistance to other classes of antimicrobial agents, such as fluoroquinolones, co-trimoxazole, and aminoglycosides,[7,8]. The resistance patterns may be multidrug resistance (MDR), extensive drug resistance (XDR) or pan drug resistance (PDR). Manu studies stated that, bacteria exhibiting MDR, XDR, and PDR are the principal organisms that undermine successful treatment [9-11]. Piperacillin-tazobactam, cefepime, aminoglycosides, trimethoprim-sulfamethoxazole and quinolones might be effective and can be considered as empirical therapy [8]. Until recently, lower UTIs were commonly treated with fluoroquinolones, resulting in a rapidly increasing prevalence of quinolone-resistant E. coli in many

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countries[12]. It is recommend to use nitrofurantoin and fosfomycin as first-line options for lower UTI in women and discourage the use of quinolones for acute cystitis because of their potential collateral damage in terms of the selection of drugresistant organisms[13]. Nitrofurantoin and fosfomycin continue to have good activity against most E. coli isolates, including ESBL producers, and have a smaller ecologic impact than the fluoroquinolones [14-16]. The current study was conducted to investigate the resistance profile for Uropathogenic E. coli and K. pneumoniae.

Materials and Methods

Sample Collection and Identification

In the present study, a total of 50 isolates of each of E. coli and K. pneumonia were collected during the period from September 2020 to January 2021, from patients with cystitis whose attended different hospitals in Hilla city, Al-Sadiq Hospital ,and Al-Hilla Teaching Hospital, Merjan Teaching city. All samples were cultured on MacConkey's and Eosin methylene blue and incubated at 37 C˚ for 24 hrs. Identification was confirmed by polymerase chain reaction (PCR) using species-specific primer pairs of E. coli (for uidA gene) and (for tyrB gene) for *K. pneumoniae* (Table 1)

Table 1. Primer pairs sequences and PCR conditions

Primer	Sequence (5′ to 3′)	Product (bp)	Annealing temp. (ºC)	Ref.
uidA-F	TGGTAATTACCGACGAAAACGGC	162	60.9	[18]
uidA-R	ACGCGTGGTTACAGTCTTGCG			
tyrB-F	GGCTGTACTACAACGATGAC	931	56.5	[19]
tyrB-F	TTGAGCAGGTAATCCACTTTG			

DNA Extraction and PCR

G-Spin Genomic DNA Extraction Kit (for bacteria) are designed isolation of genomic DNA from a variety of sample sources including fresh or frozen animal cells and Gram negative bacteria. Conventional PCR was used to amplify the target DNA using specific primer pairs (Table 1). The PCR condition is illustrated in Table 2 for the PCR mixture of 20µl consisted of 5 µl of Maxime PCR Premix kit (i-Taq) (Intronbio/Korea), 2.5 µl of forward primer (10(pmole/µl), 2.5 µl of reverse primer (10 pmole/µl), (5 µl) of target DNA, and 5µl of nuclease-free waterNew Biolabs/US.

Antibacterial Susceptibility Test

The in vitro susceptibility ofEnterobateraciaspp isolates to 26 antibiotics was determined by disc-diffusion method according to the clinical and laboratory standards institute (CLSI, 2019) [17] Activation of isolates was done using the nutrient broth for 18 h at37C and the growth was adjusted to 0.5 McFarland's standard (1.5×108 CFU/mL), then spread on Mueller Hinton agar (MHA) with a cotton swab. Antibiotic discs were placed on MHA, gently pressed down to ensure complete contact with the agar inoculated with bacteria, and then incubated for 18–20 h at 37°C and then inhibition zone diameter in mm was recorded.Interpretation of results as a sensitive or resist was achieved according to the CLSI, 2019.[17]

Results and Discussion

The results revealed that 50 isolates were lactose fermenter with green metallic sheen on EMB while the other 50 isolates were lactose fermenter with deep purple mucoid appearance on EMB. The first screening on EMB were confirmed using species specific primers (uidA gene) for E. coli and (tyrB gene) for K. pneumoniae (Figure 1 and 2).β-D-glucuronidase, encoded by the gene uidA, is routinely used to specifically identify *E. coli*. Many studies revealed that uidA was found in about 97% of all strains of E. coli. This gene encodes an enzyme specific to *E. coli* and is therefore widely used in identification kits and

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as a specific marker for *E. coli* [20-23]. It was found that uidApresent in of ETEC [24], E. coli isolated from meat[25], diarrhoea[26-28], wastewater [29], pigs [30], cattle [31]. Concern using of tyrB gene (which encode for Tyrosine aminotransferase) for specific identification of K. pneumoniae, many studies proved their specificity for identification of this bacteria isolated from different clinical samples [32-35].

Concern antibiotic susceptibility of E. coli, for penicillin and cephalosporin the results revealed that it is fully resist to amoxicillin, ceftazidime and cefotaxime while express moderate resistance to ceftriaxone (54%), cefixime (52%) and Cefoxitin (42%). The isolates express high sensitivity for piperacillin (74%) (figure 3). Also the susceptibility results to monobactam and carbapenem revealed that, (20%, 0.00% and 6%) were resistant to Aztreonam, imipenem and meropenem respectively while high sensitivity were documented (96%, 80% and 56%) for imipenem and meropenem, Aztreonam respectively (figure 4). For quinolones, the isolates show low resistance (26%, 14% and 12%) for ciprofloxacin , ofloxacine and levofloxacin respectively. At same time they are express high sensitivity rate (82% and 80%) to ofloxacin and levofloxacin respectively (figure 5). Susceptibility of E. coli to aminoglycosides revealed that moderate resistance to kanamycin , streptomycin and tobramycin (42%, 36% and 20% respectively). High sensitivity were stated (94%, 62%, 50%, 50%) to netilmicine, gentamycin amikacin and tobramycin respectively (figure 6). Also the isolates show high sensitivity to azithromycin (90%), trimethoprim (64%), doxycycline (62%) and nalidixic acid (62%) (figure 7). It seem that the E. coli isolates have extend spectrum beta-lactamases due to their resistance to ceftazidime, cefotaxime and moderately to ceftriaxone. This is may be attributed to blaCTX-M type especially (blaCTX-M-15. blaCTX-M-27)[36], blaCTX-M-1, blaCTX-M-2, blaCTX-M-8, blaCTX-M-9[37], bla-TEM and bla-SHV [38,39], blaOXA and AmpC[40]. Resistance to aminoglycosides may be mediated by modifying enzymes like aac(6′)-Ib, aac(3)-II, *ant(3")-I* (8.4%) and *aph(3')-VI* [41,42]. Carbapenem-resistant E. coli isolates may be due to blaKPC-2 and blaNDM-1[43]. Additionally the resistance to more than one antibiotics may be attributed to efflux pumps [45]. AcrAB-TolC was the predominant efflux pump for most of antibiotic classes [46-48]. EmrAB-TolC was also staed as more prevalent efflux pump of UPEC[49]

The results of K. pneumoniae susceptibility revealed that high resistance to penicillins and cephalosporins (ceftazidime 98%, ceftriaxone 92%, cefotaxime 92%, amoxicillin 92%, cefixime 84% and cefepime 80%). It is only sensitive to piperacillin (66%) (figure 8). Additionally the isolates express high sensitivity to meropenem (86%), and imipenem (76%) while moderate resistance to Aztreonam (58%) (figure 9). Quinolones susceptibility results reveal that, they are highly sensitive to ofloxacin (90%) and moderate sensitivity to levofloxacin (58%) and ciprofloxacin (46%) (figure 10). High level of sensitivity were conveyed to netlimicin (92%), amikacin (82%) and moderate sensitivity to gentamycin (52%) while high resistance was for streptomycin (78%)(figure 11). They show high sensitivity to azithromycin (76%) and nalidixic acid (68%) while express high resistance to trimethoprim (76%) and nitrofurantion (58%) (figure 12). Resistance of K. pneumoniae to beta-lactam also may be mediated by *beta-lactamases like blaOXA-48 and blaKPC*[50]. CTX-M-15 was the most frequent ESBL identified in K. pneumoniae[51]. blaAmpC was also detected in K. pneumoniae[52,53]. ESBL producers represented one-third of E. coli/K. pneumoniae UTI episodes[54]. Resistance to quinolone may be mediated by qnrS, qnrB and qnrA . The most prevalent quinolone efflux pump gene was oqxB , followed by oqxA and qepA [55]. It was found that ciprofloxacin-resistant strains of K. pneumoniae have overexpressed AcrAB, MacAB and OxqAB-TolC efflux pump genes[56-59]. Current treatment options for UTIs due to ESBL-producing Enterobacteriales include nitrofurantoin, fosfomycin, fluoroquinolones and carbapenems[60].

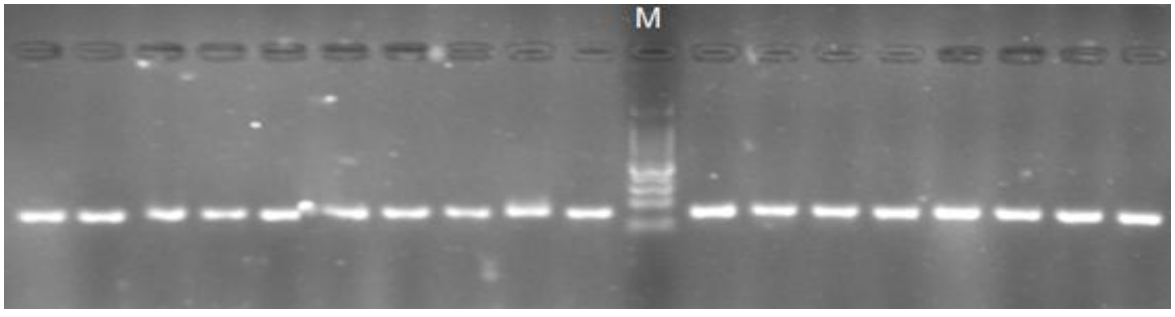

Figure 1. Agarose gel electrophoresis (1.5% in TBE) for *uidA* amplicon (162bp), M represent 100bp DNA marker , other lanes represent E. coli isolates

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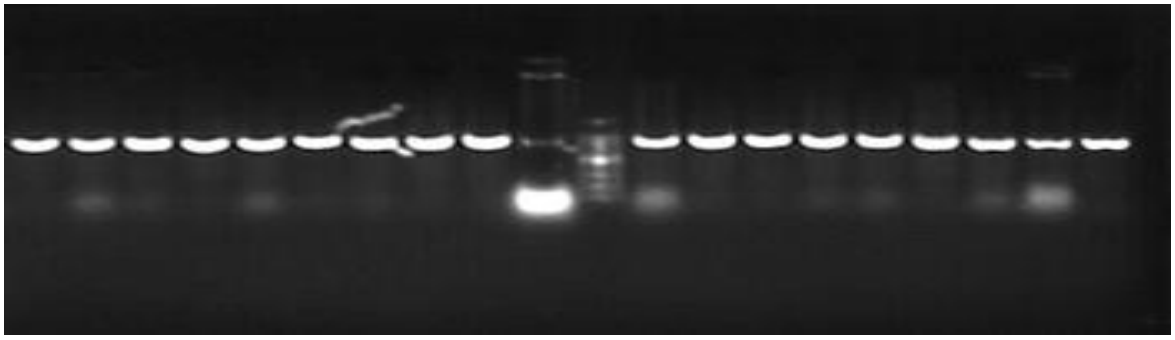

Figure 2. Agarose gel electrophoresis (1.5% in TBE) for *tyrB* amplicon (931bp), M represent 100bp DNA marker , other lanes represent K. pneumoniae isolates

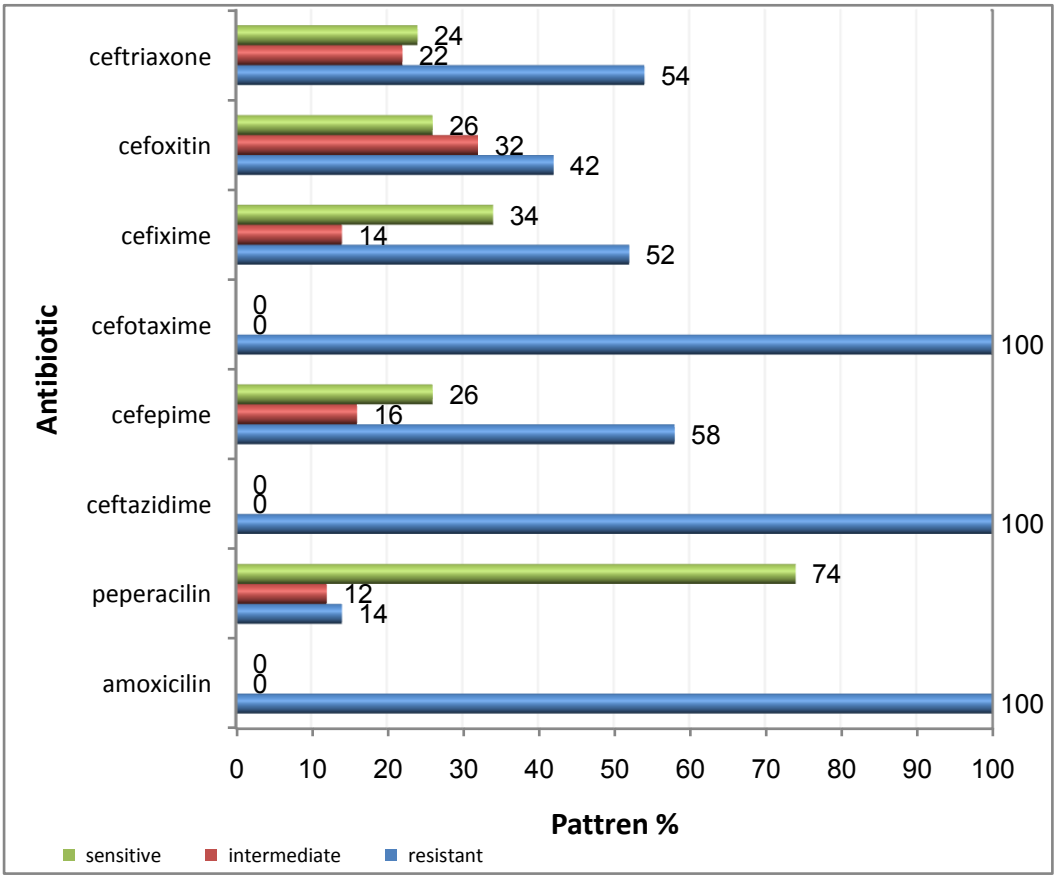

Figure 3. Antibiotic resistance patterns % of E. coli for penicillins and cephalosporins

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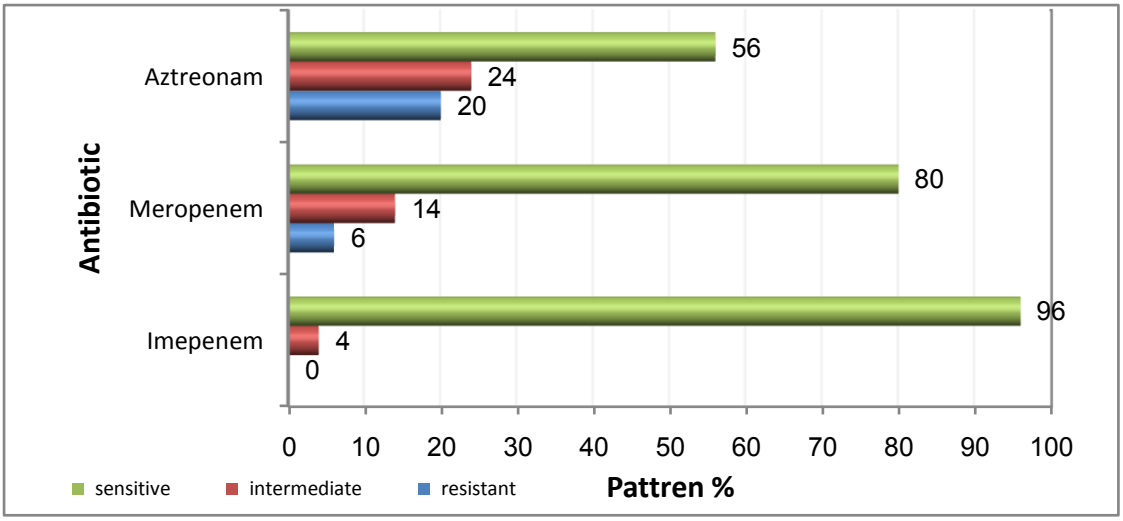

Figure 4. Antibiotic resistance patterns % of E. coli for monobactam and carbapenem

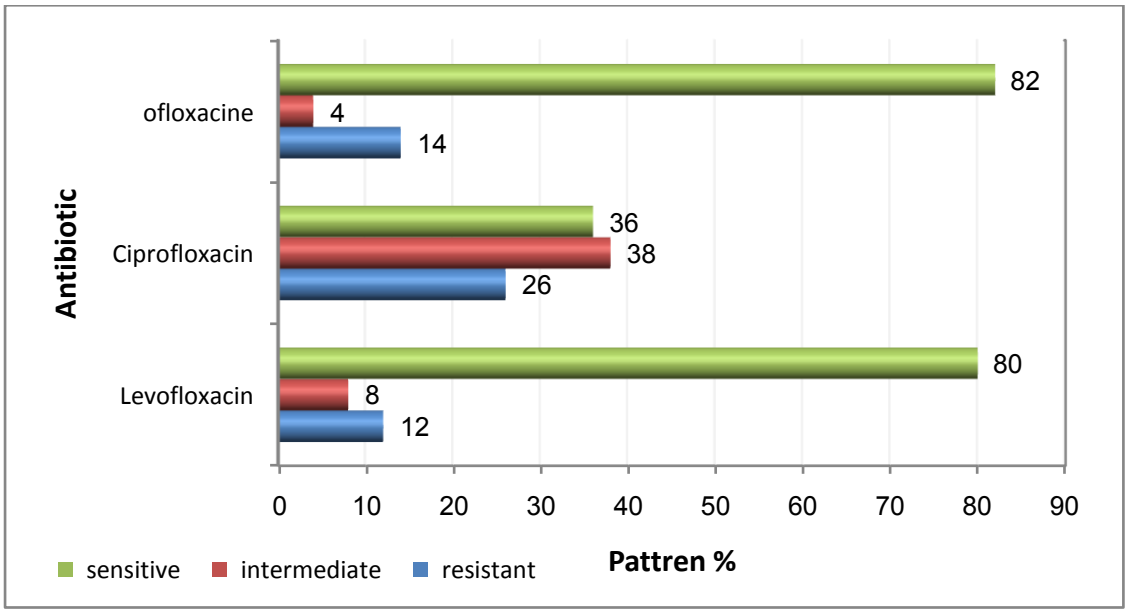

Figure 5. Antibiotic resistance patterns % of E. coli for quinolones

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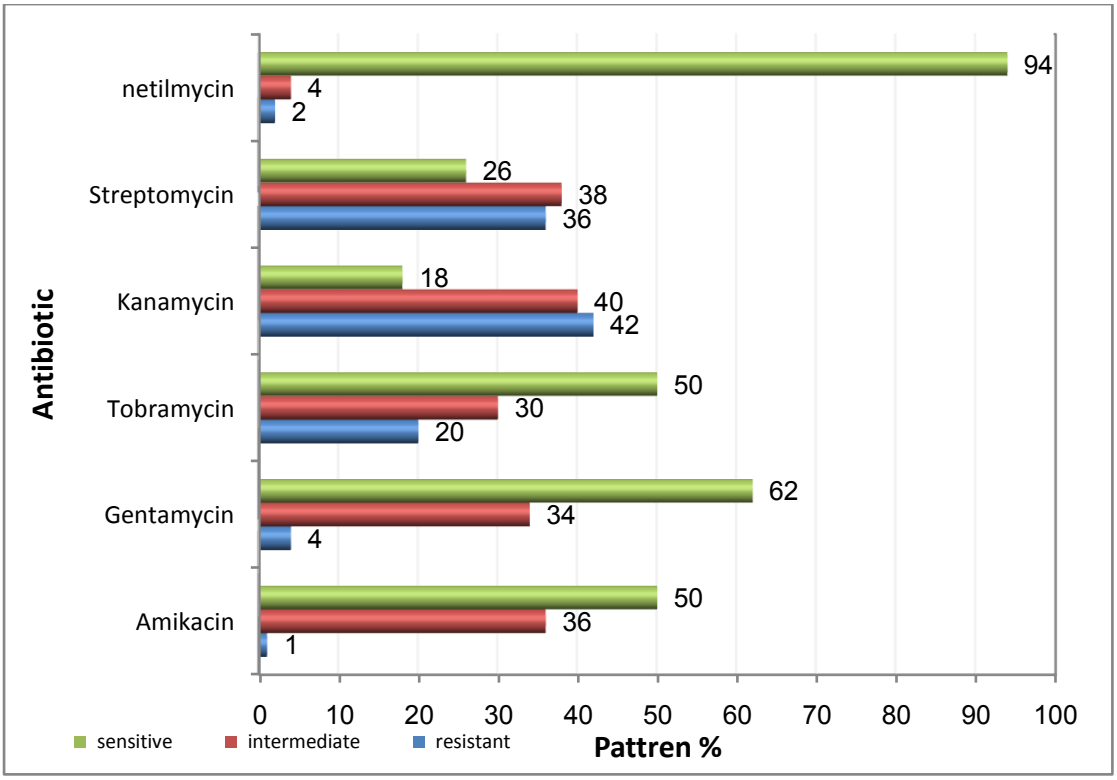

Figure 6. Antibiotic resistance patterns % of E. coli for aminoglycosides

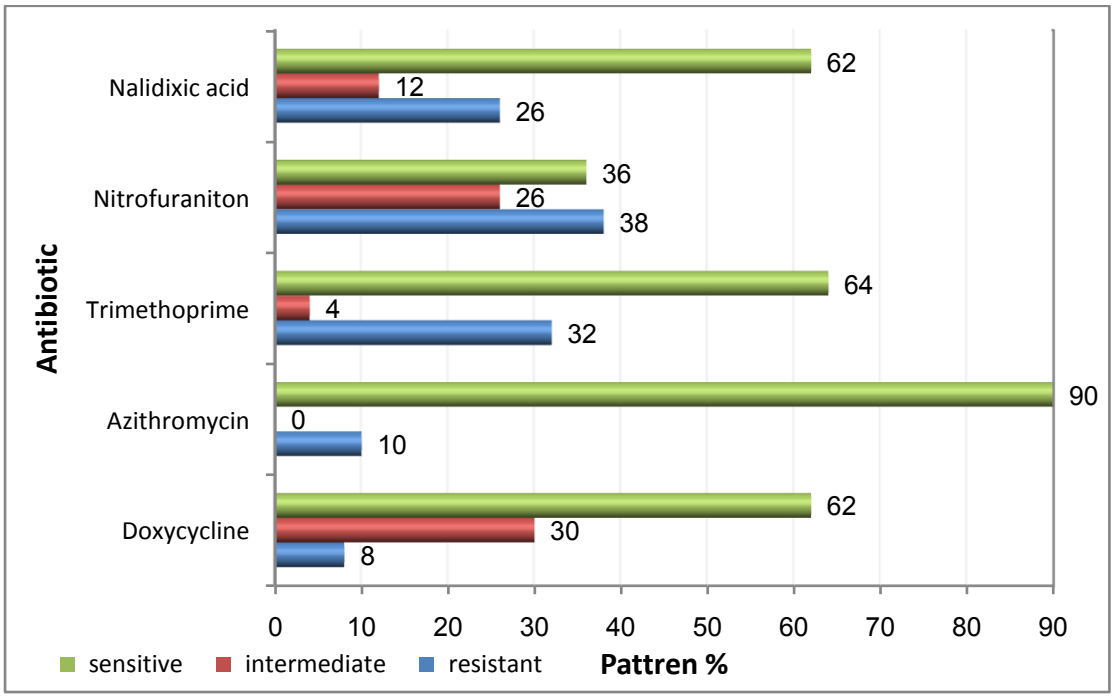

Figure 7. Antibiotic resistance patterns % of E. coli for other classes of antibiotics

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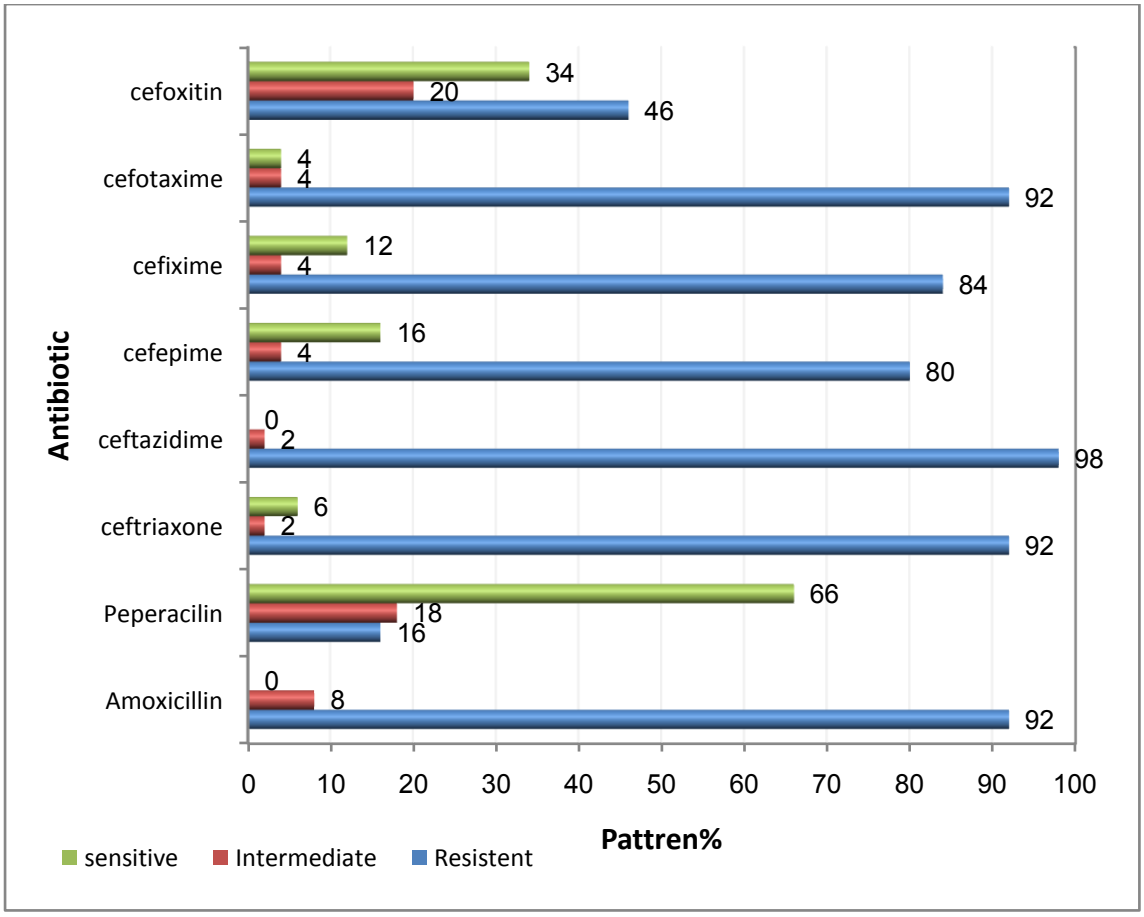

Figure 8. Antibiotic resistance patterns % of K. pneumoniae for penicillins and cephalosporins

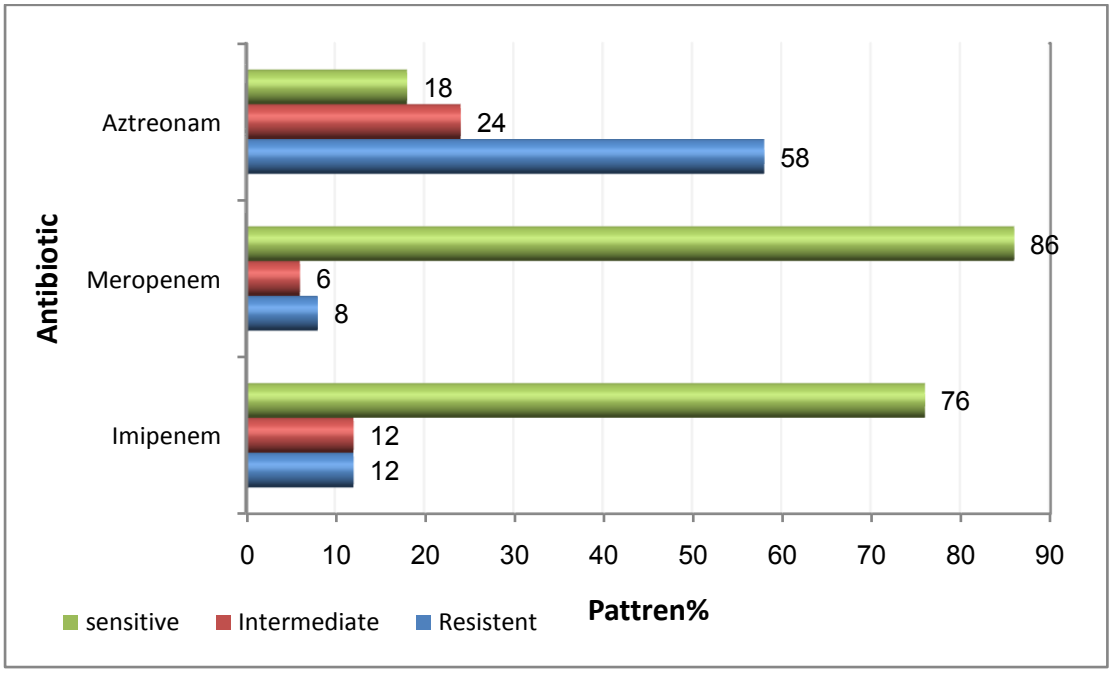

Figure 9. Antibiotic resistance patterns % of K. pneumoniae for monobactam and carbapenem

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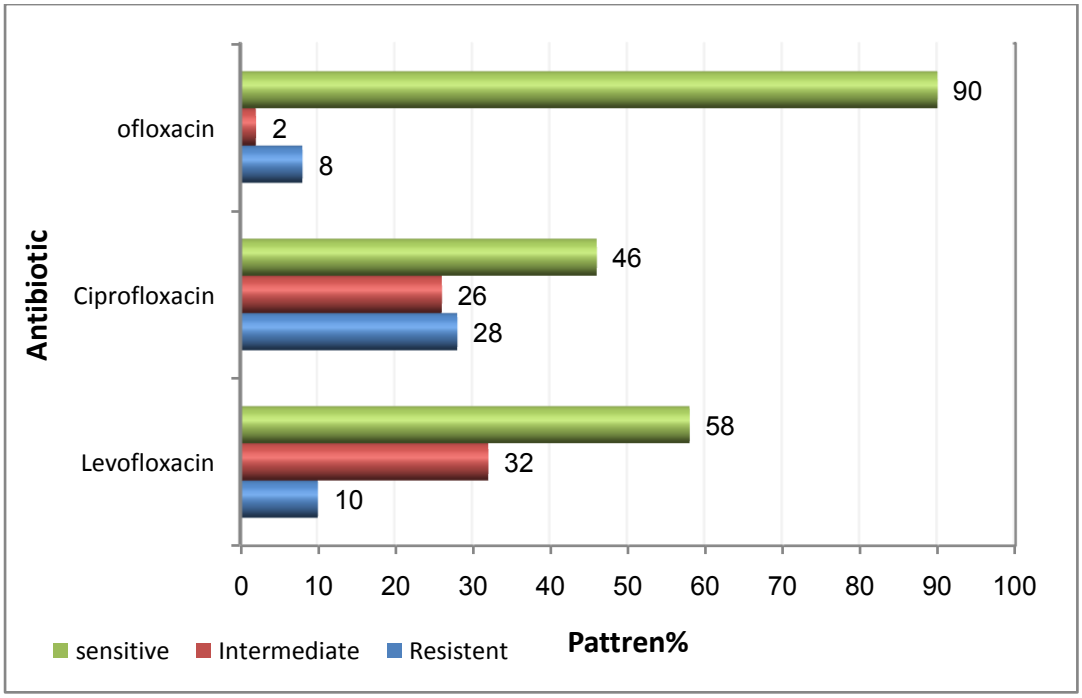

Figure 10. Antibiotic resistance patterns % of K. pneumoniae for quinolones

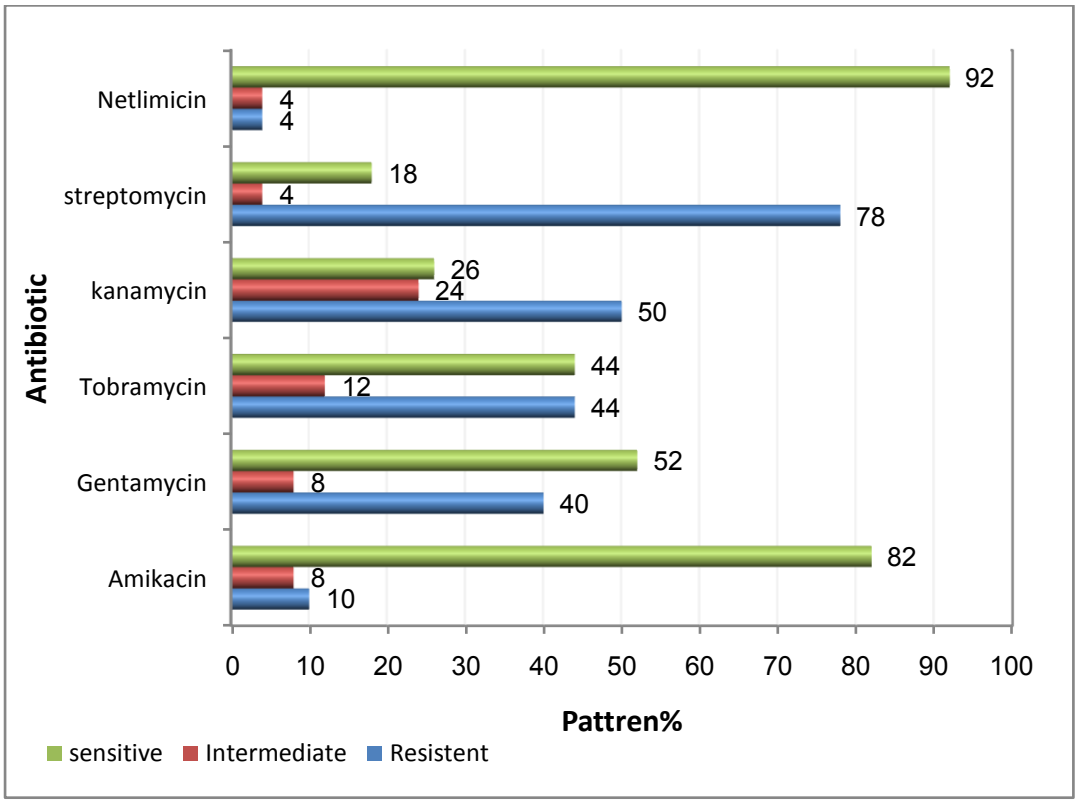

Figure 11. Antibiotic resistance patterns % of K. pneumoniae for aminoglycosides

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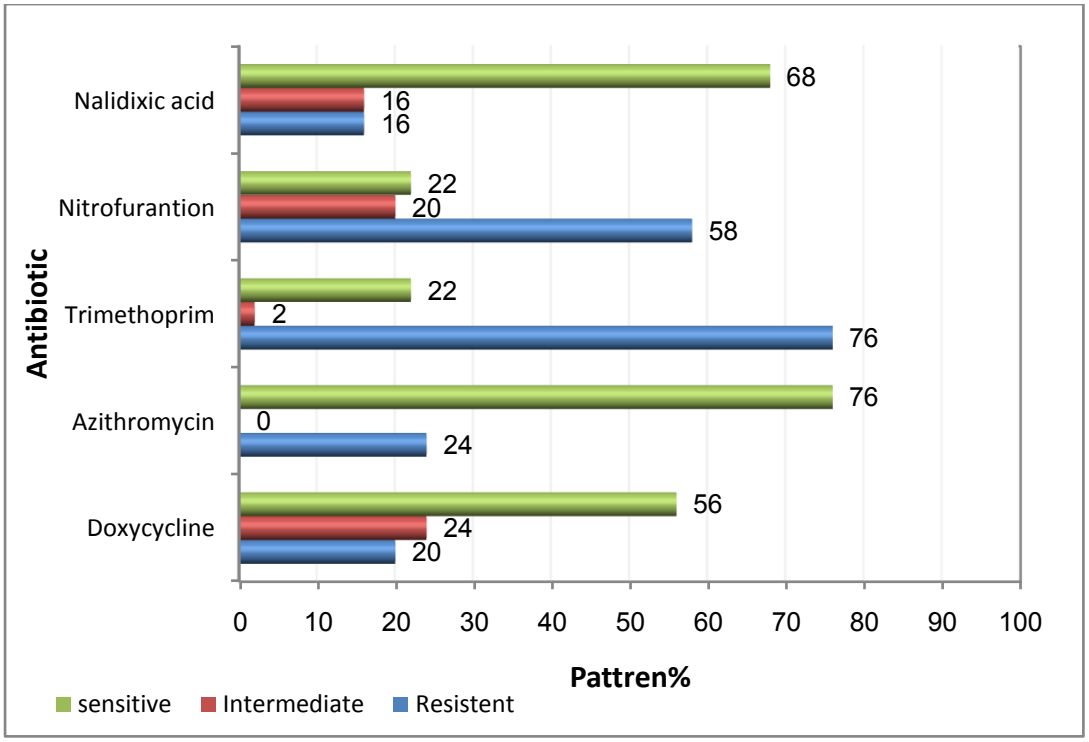

Figure 12. Antibiotic resistance patterns % of K. pneumoniae for other classes of antibiotics

Conclusion:

The current study conclude that, the most suitable drugs for UTIs-associated E. coli/K. pneumoniae were piperacillin, netilmicine, amikacin, imipenem, meropenem, levofloxacin. There is an increased emergence of resistance to cephalosporins along nitrofurantion and trimethoprime.

References

[1] Flores-Mireles, A. L., Walker, J. N., Caparon, M., &Hultgren, S. J. (2015). Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nature reviews microbiology, 13(5), 269-284.
[2] Magill, S. S., Hellinger, W., Cohen, J., Kay, R., Bailey, C., Boland, B., and Fridkin, S. (2012). Prevalence of healthcare-associated infections in acute care hospitals in Jacksonville, Florida. Infection control and hospital epidemiology, 33(3), 283.
[3] Stultz, J. S., Doern, C. D., & Godbout, E. (2016). Antibiotic resistance in pediatric urinary tract infections. Current infectious disease reports, 18(12), 1-9.
[4] Gupta, K. (2003). Emerging antibiotic resistance in urinary tract pathogens. Infectious disease clinics of North America, 17(2), 243-259.
[5] Zilberberg, M. D., Nathanson, B. H., Sulham, K., Fan, W., &Shorr, A. F. (2017). Carbapenem resistance, inappropriate empiric treatment and outcomes among patients hospitalized with Enterobacteriaceae urinary tract infection, pneumonia and sepsis. BMC infectious diseases, 17(1), 1-13.
[6] Khoshnood, S., Heidary, M., Mirnejad, R., Bahramian, A., Sedighi, M., &Mirzaei, H. (2017). Drug-resistant gram-negative uropathogens: A review. Biomedicine & Pharmacotherapy, 94, 982-994.
[7] Falagas, M. E., Polemis, M., Alexiou, V. G., Marini-Mastrogiannaki, A., Kremastinou, J., &Vatopoulos, A. C. (2008). Antimicrobial resistance of Esherichia coli urinary isolates from primary care patients in Greece. Medical Science Monitor, 14(2), CR75-CR79.
[8] Moxon, C. A., & Paulus, S. (2016). Beta-lactamases in Enterobacteriaceae infections in children. Journal of infection, 72, S41-S49.
[9] Basak, S., Singh, P., &Rajurkar, M. (2016). Multidrug resistant and extensively drug resistant bacteria: A study. Journal of pathogens, 2016.

[10] Gill, J. S., Arora, S., Khanna, S. P., & Kumar, K. H. (2016). Prevalence of multidrug-resistant, extensively drug-resistant, and pandrug-resistant Pseudomonas aeruginosa from a tertiary level intensive care unit. Journal of global infectious diseases, 8(4), 155.
[11] Beyene, D., Bitew, A., Fantew, S., Mihret, A., & Evans, M. (2019). Multidrug-resistant profile and prevalence of extended spectrum β-lactamase and carbapenemase production in fermentative Gram-negative bacilli recovered from patients and specimens referred to National Reference Laboratory, Addis Ababa, Ethiopia. PloS one, 14(9), e0222911.
[12] Blaettler, L., Mertz, D., Frei, R., Elzi, L., Widmer, A. F., Battegay, M., &Flückiger, U. (2009). Secular trend and risk factors for antimicrobial resistance in Escherichia coli isolates in Switzerland 1997–2007. Infection, 37(6), 534.
[13] Gupta, K., Hooton, T. M., Naber, K. G., Wullt, B., Colgan, R., Miller, L. G., ... &Soper, D. E. (2011). International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: a 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clinical infectious diseases, 52(5), e103-e120.
[14] Bonkat, G., Müller, G., Braissant, O., Frei, R., Tschudin-Suter, S., Rieken, M., ... &Widmer, A. F. (2013). Increasing prevalence of ciprofloxacin resistance in extended-spectrum-β-lactamase-producing Escherichia coli urinary isolates. World journal of urology, 31(6), 1427-1432.
[15] Sanchez GV, Baird AM, Karlowsky JA, Master RN, Bordon JM. Nitrofurantoin retains antimicrobial activity against multidrug-resistant urinary Escherichia coli from US outpatients. Journal of Antimicrobial Chemotherapy. 2014 Dec 1;69(12):3259-62.
[16] Stewardson, A. J., Vervoort, J., Adriaenssens, N., Coenen, S., Godycki-Cwirko, M., Kowalczyk, A., ... &Żebrowska, L. (2018). Effect of outpatient antibiotics for urinary tract infections on antimicrobial resistance among commensal Enterobacteriaceae: a multinational prospective cohort study. Clinical Microbiology and Infection, 24(9), 972-979.
[17] CLSI. Performance standards for antimicrobial susceptibility testing. 29ed. CLSI supplement M100-S29. Wayne, PA: Clinical and laboratory standards institute;2020.
[18] Bej, A. K., Dicesare, J. L., Haff, L., & Atlas, R. M. (1991). Detection of Escherichia coli and Shigella spp. in water by using the polymerase chain reaction and gene probes for uid. Applied and Environmental Microbiology, 57(4), 1013-1017.
[19] JEong ES, Lee KS, HEo SH, SEo JH, CHoi YK. Rapid identification of Klebsiella pneumoniae, Corynebacterium kutscheri, and Streptococcus pneumoniae using triplex polymerase chain reaction in rodents. Experimental animals. 2013;62(1):35-40.
[20] Lasalde, C., Huertas, A., Rodrıguez, R., &Toranzos, G. A. (2003). Escherichia coli populations and uidA gene variation in atropical rain forest soil. 103rd American Society for Microbiology (ASM) General Meeting. Washington, DC. pp, 18-22.
[21] Johnson, J. R., Johnston, B. D., & Gordon, D. M. (2017). Rapid and specific detection of the Escherichia coli sequence type 648 complex within phylogroup F. Journal of clinical microbiology, 55(4), 1116-1121.
[22] Kiel, M., Sagory-Zalkind, P., Miganeh, C., Stork, C., Leimbach, A., Sekse, C., ... &Dobrindt, U. (2018). Identification of novel biomarkers for priority serotypes of Shiga toxin-producing Escherichia coli and the development of multiplex PCR for their detection. Frontiers in microbiology, 9, 1321.
[23] Godambe, L. P., Bandekar, J., &Shashidhar, R. (2017). Species specific PCR based detection of Escherichia coli from Indian foods. 3 Biotech, 7(2), 1-5.
[24] Chigor, V., Ibangha, I. A., Chigor, C., &Titilawo, Y. (2020). Treated wastewater used in fresh produce irrigation in Nsukka, Southeast Nigeria is a reservoir of enterotoxigenic and multidrug-resistant Escherichia coli. Heliyon, 6(4), e03780.
[25] Tshabalala R, Kabelinde A, Foka FE, Ateba CN, Manganyi MC. Effect of Clove (Syzygiumaromaticum) spice as microbial inhibitor of resistant bacteria and Organoleptic Quality of meat. Saudi Journal of Biological Sciences. 2021 Mar 27.
[26] Bhardwaj, P., Kaur, G., &Rampal, S. (2020). Impact of marbofloxacin administration on the emergence of marbofloxacin-resistant E. coli in faecal flora of goats and elucidation of molecular basis of resistance. Journal of global antimicrobial resistance, 21, 116-123.
[27] Omolajaiye, S. A., Afolabi, K. O., &Iweriebor, B. C. (2020). Pathotyping and Antibiotic Resistance Profiling of Escherichia coli Isolates from Children with Acute Diarrhea in Amatole District Municipality of Eastern Cape, South Africa. BioMed Research International, 2020.
[28] Msolo, L., Iweriebor, B. C., &Okoh, A. I. (2020). Pervasiveness of diarrheagenic E. coli pathotypes and Salmonella species among gastroenteritis patients in some selected pastoral hinterlands of the Amathole district municipality, Eastern Cape, South Africa. Human Microbiome Journal, 17, 100074.
[29] Adekanmbi, A. O., Akinpelu, M. O., Olaposi, A. V., &Oyelade, A. A. (2020). Extended spectrum beta-lactamase encoding gene-fingerprints in multidrug resistant Escherichia coli isolated from wastewater and sludge of a hospital treatment plant in Nigeria. International Journal of Environmental Studies, 1-11.

[30] Abdalla, S. E., Abia, A. L. K., Amoako, D. G., Perrett, K., Bester, L. A., &Essack, S. Y. (2021). From farm-to-fork: E. coli from an intensive pig production system in South Africa shows high resistance to critically important antibiotics for human and animal use. Antibiotics, 10(2), 178.
[31] Debbarma, S., Bora, D. P., Hazarika, R. A., Tamuly, S., Barua, A. G., Das, P., ... &Sinha, S. (2020). Molecular characterization of Escherichia Coli isolates from food animals. Indian Journal of Animal Research, 54(8), 985-993.
[32] Dong, D., Liu, W., Li, H., Wang, Y., Li, X., Zou, D., ... & Yuan, J. (2015). Survey and rapid detection of Klebsiella pneumoniae in clinical samples targeting the rcsA gene in Beijing, China. Frontiers in microbiology, 6, 519.
[33] JEong, E. S., Lee, K. S., HEo, S. H., SEo, J. H., &CHoi, Y. K. (2013). Rapid identification of Klebsiella pneumoniae, Corynebacterium kutscheri, and Streptococcus pneumoniae using triplex polymerase chain reaction in rodents. Experimental animals, 62(1), 35-40.
[34] Yin-Ching, C., Jer-Horng, S., Ching-Nan, L., & Ming-Chung, C. (2002). Cloning of a gene encoding a unique haemolysin from Klebsiella pneumoniae and its potential use as a species-specific gene probe. Microbial pathogenesis, 33(1), 1-6.
[35] Onanuga, A., Eboh, D. D., Odetoyin, B., &Adamu, O. J. (2020). Detection of ESBLs and NDM-1 genes among urinary Escherichia coli and Klebsiella pneumoniae from healthy students in Niger Delta University, Amassoma, Bayelsa State, Nigeria. PAMJ-One Health, 2(12).
[36] Birgy, A., Madhi, F., Jung, C., Levy, C., Cointe, A., Bidet, P., ... &Bonacorsi, S. (2020). Diversity and trends in population structure of ESBL-producing Enterobacteriaceae in febrile urinary tract infections in children in France from 2014 to 2017. Journal of Antimicrobial Chemotherapy, 75(1), 96-105.
[37] Hassuna, N. A., Khairalla, A. S., Farahat, E. M., Hammad, A. M., & Abdel-Fattah, M. (2020). Molecular characterization of Extended-spectrum β lactamase-producing E. coli recovered from community-acquired urinary tract infections in Upper Egypt. Scientific reports, 10(1), 1-8.
[38] Pandit, R., Awal, B., Shrestha, S. S., Joshi, G., Rijal, B. P., &Parajuli, N. P. (2020). Extended-spectrum β-lactamase (ESBL) genotypes among multidrug-resistant uropathogenic Escherichia coli clinical isolates from a teaching hospital of Nepal. Interdisciplinary perspectives on infectious diseases, 2020.
[39] Naziri, Z., Derakhshandeh, A., Borchaloee, A. S., Poormaleknia, M., &Azimzadeh, N. (2020). Treatment Failure in Urinary Tract Infections: A Warning Witness for Virulent Multi-Drug Resistant ESBL-Producing Escherichia coli. Infection and drug resistance, 13, 1839.
[40] Gajamer, V. R., Bhattacharjee, A., Paul, D., Ingti, B., Sarkar, A., Kapil, J., ... &Tiwari, H. K. (2020). High prevalence of carbapenemase, AmpC β-lactamase and aminoglycoside resistance genes in extended-spectrum β-lactamase-positive uropathogens from Northern India. Journal of global antimicrobial resistance, 20, 197-203.
[41] Esmaeel, N. E., Gerges, M. A., Hosny, T. A., Ali, A. R., &Gebriel, M. G. (2020). Detection of Chromosomal and Plasmid-Mediated Quinolone Resistance Among Escherichia coli Isolated from Urinary Tract Infection Cases; Zagazig University Hospitals, Egypt. Infection and drug resistance, 13, 413.
[42] Sadeghi, M., Ebrahim-Saraie, H. S., Mojtahedi, A., Nikokar, I., &Roushan, Z. A. (2020). Genetic diversity and prevalence of aminoglycoside modifying enzymes among Escherichia coli strains isolated from inpatients with urinary tract infections. Gene Reports, 21, 100957.
[43] De La Cadena, E., Mojica, M. F., Castillo, N., Correa, A., Appel, T. M., García-Betancur, J. C., ... & Villegas, M. V. (2020). Genomic Analysis of CTX-M-Group-1-Producing Extraintestinal Pathogenic E. coli (ExPEc) from Patients with Urinary Tract Infections (UTI) from Colombia. Antibiotics, 9(12), 899.
[45] Al-Zuhairy, A. M., & Al-Dahmoshi, H. O. (2020). Aminoglycosides and fluoroquinolones resistant genes among uropathogenic Escherichia coli in Hilla City, Iraq. Drug Invention Today, 13(2).
[46] Awadallah, M. G., Amer, G. A., Emam, S. M., & Ramadan, A. E. Multidrug Efflux Pump In Relation To Antibiotic Resistance Pattern in Escherichia Coli Strains Isolated From Benha University Hospital.
[47] Mulakhudair, A. R., Hadi, K. A., &Mulakhudair, A. R. (2020). Isolation, identification and detection of some acriflavine (Acr) Efflux pumps genes in uropathogenic E. coli isolates isolated from Iraqis patients. Annals of Tropical Medicine and Health, 23, 232-119.
[48] Fayyazi, A., Halaji, M., Sadeghi, A., &Havaei, S. A. (2020). High frequency of integrons and efflux pump in uropathogenic Escherichia coli isolated from Iranian kidney and non-kidney transplant patients. Gene Reports, 21, 100873.
[49] Yousefian, N., Ornik-Cha, A., Poussard, S., Decossas, M., Berbon, M., Daury, L., ... &Pos, K. M. (2021). Structural characterization of the EmrAB-TolC efflux complex from E. coli. *Biochimica et BiophysicaActa (BBA)-Biomembranes*, *1863*(1), 183488.
[50] [C] Gurung, S., Kafle, S., Dhungel, B., Adhikari, N., Shrestha, U. T., Adhikari, B., ... &Ghimire, P. (2020). Detection of OXA-48 gene in carbapenem-resistant Escherichia coli and Klebsiella pneumoniae from urine samples. Infection and Drug Resistance, 13, 2311.

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[51] Fils, P. E. L., Cholley, P., Gbaguidi-Haore, H., Hocquet, D., Sauget, M., & Bertrand, X. (2021). ESBL-producing Klebsiella pneumoniae in a University hospital: Molecular features, diffusion of epidemic clones and evaluation of cross-transmission. Plos one, 16(3), e0247875.

[52] Kurittu, P., Khakipoor, B., Aarnio, M., Nykäsenoja, S., Brouwer, M., Myllyniemi, A. L., ... &Heikinheimo, A. (2021). Plasmid-borne and chromosomal ESBL/AmpC genes in Escherichia coli and Klebsiella pneumoniae in global food products. Frontiers in Microbiology, 12.

[53] Xiong, Y., Zhang, C., Gao, W., Ma, Y., Zhang, Q., Han, Y., ... & Chen, Y. (2021). Genetic diversity and co-prevalence of ESBLs and PMQR genes among plasmid-mediated AmpC β-lactamase-producing Klebsiella pneumoniae isolates causing urinary tract infection. The Journal of Antibiotics, 1-10.

[54] Vachvanichsanong, P., McNeil, E. B., &Dissaneewate, P. (2021). Extended-spectrum beta-lactamase Escherichia coli and Klebsiella pneumoniae urinary tract infections. Epidemiology & Infection, 149.

[55] Elshamy, A. A., Aboshanab, K. M., Yassien, M. A., &Hassouna, N. A. (2020). Prevalence of plasmid-mediated resistance genes among multidrug-resistant uropathogens in Egypt. African Health Sciences, 20(1), 190-198.

[56] Dolatabadi, A., Noorbazargan, H., Khayam, N., Moulavi, P., Zamani, N., AsghariLalami, Z., &Ashrafi, F. (2021). Ecofriendly Biomolecule-Capped Bifidobacteriumbifidum-Manufactured Silver Nanoparticles and Efflux Pump Genes Expression Alteration in Klebsiella pneumoniae. Microbial Drug Resistance, 27(2), 247-257.

[57] Xu, Q., Sheng, Z., Hao, M., Jiang, J., Ye, M., Chen, Y., ... & Wang, M. (2021). RamAupregulates multidrug resistance efflux pumps AcrAB and OqxAB in Klebsiella pneumoniae. International Journal of Antimicrobial Agents, 57(2), 106251.

[58] Zheng, J. X., Lin, Z. W., Sun, X., Lin, W. H., Chen, Z., Wu, Y., ... & Yu, Z. J. (2018). Overexpression of OqxAB and MacAB efflux pumps contributes to eravacycline resistance and heteroresistance in clinical isolates of Klebsiella pneumoniae. Emerging microbes & infections, 7(1), 1-11.

[59] Lee, Y. J., Huang, C. H., Ilsan, N. A., Lee, I. H., & Huang, T. W. (2021). Molecular Epidemiology and Characterization of Carbapenem-Resistant Klebsiella pneumoniae Isolated from Urine at a Teaching Hospital in Taiwan. Microorganisms, 9(2), 271.

[60] Bader, M. S., Loeb, M., Leto, D., & Brooks, A. A. (2020). Treatment of urinary tract infections in the era of antimicrobial resistance and new antimicrobial agents. Postgraduate medicine, 132(3), 234-250.