Saudi Journal of Medical and Pharmaceutical Sciences **ISSN 2413-4929 (Print)**
Scholars Middle East Publishers **ISSN 2413-4910 (Online)**
Dubai, United Arab Emirates
Website: http://saudijournals.com/

Extended-spectrum Beta-lactamase- Producing *Escherichia coli* and *Klebsiella pneumoniae* Clinical Isolates in Sudanese Hospitals: Analytical Comparative Cross Sectional Study

Hitham Mohammed[1*], Ahmed Mohammed Ahmed[1], Saeed Abdelgadir Saeed[2], Ahmed Musa Abdalla[3], Nuraldeen Mohammed Ali[4]
[1]Pathology department, Shendi University, Sudan
[2]Medical laboratory department, Eastern Nile University College, Sudan
[3]Tumor diagnoses Clinic, Sudan
[4]Public health laboratory, Laboratory administration, Khartoum State, Sudan

Original Research Article

***Corresponding author**
Hitham Mohammed.
E-mail:
abumohammedosman2020@gmail.com

Article History
Received: 02.11.2018
Accepted: 07.11.2018
Published: 30.11.2018

DOI:
10.36348/sjmps.2018.v04i11.006

Abstract: Extended-spectrum – beta lactamases (ESBLs) are increasingly detected globally among *Escherichia coli (E.Coli)* and *Klebsiella pneumoniae (K.pneumoniae).* This study was designed to determine the prevalence and antibiogram of ESBLs produced by *E.coli* and *K.pneumoniae* clinical isolates obtained from various clinical specimens through different hospitals in Khartoum state -Sudan. An analytical comparative cross-sectional study was conducted. Identification of the isolates was done by using conventional biochemical methods ESBL screening, confirmatory according to CLSI guidelines. Statistical analysis was performed using the Statistical Package of Social Sciences (SPSS). Total of 368 isolates of *Escherichia coli*(n=216) and *Klebsiella pneumonia* (n=152) isolates were processed , Overall ESBL phenotype prevalence was 36.7 %, 40.1% and 34.2% of *K. Pneumoniae* and *E. coli* isolates respectively were confirmed to be ESBL phenotype. In this study, meropenem and imipenem were the most active antimicrobial agents against them. This study was indicated high prevalence of ESBL phenotype in Khartoum-state, with multidrug resistant trend, there is a need for longitudinal and nationwide surveillance as this help in tracking antibiotic resistant and regulating antibiotic policy.

Keywords: ESBL, Khartoum state, MDR, *Escherichia coli*, *Klebsiella pneumoniae*.

INTRODUCTION

β lactamases are bacterial enzymes that inactivate β-lactam antibiotics by hydrolysis, which results in active compounds. One group of β lactamases, extended-spectrum β lactamases (ESBLs), Extended spectrum β lactamases (ESBLs) bacteria are emerging worldwide as a threat to favorable outcome in the treatment of common infections in community and hospital settings [1, 2].

ESBLs are firstly reported in 1983 from Germany, a commonly used working definition is that the ESBLs are β-lactamases capable of conferring bacterial resistance to all penicillins, first-to-fourth generation cephalosporins and monobactams, but not to cephamycins or carbapenems [3].

E. coli, K. pneumoniae, are the most common ESBL-producing bacterial species,The presence of ESBL in these two strains ,poses a great challenge in clinical practice, since these organisms are common causes of serious infections [4]. Imipenem and meropenem are considered the therapy of choice for patients with serious infections due to ESBL producing strains [5].

ESBLs genotypes: TEM, SHV, and CTX-M are the most prevalent,the SHV enzymes are named abbreviations after Sulphydryl Variable site, the TEM enzyme was named after the patient from which it was derived, Temoneira [6].

CTX-M as its predominant cefotaximase activity and the location of its isolation (Munich) the enzyme was named , CTX-M ESBLs are subdivided into five groups on the basis of amino acid sequence

similarity: CTX-M-1 group, CTX-M-2 group, CTX-M-8 group, CTX-M-9 group, and CTX-M-25 group [7].

In African countries, there is generally a lack of comprehensive data with regards to ESBLs, in Ethiopia, 38.4% (n=43) of *E. coli* and K. pneumonae isolates were ESBL producer , in Kenya Hospital and community based study showed that 37.4% of the isolate were ESBL producer, in Sudan Hospital based prospective study conducted in 2011 showed that among *E. coli* isolates 30.2%(n= 70) were ESBL producer, in Eygpt The prevalence of ESBL producing Enterobactericea ranges from (11-42.9%) in both hospital and community [8].

Prevalence of ESBLs varies from institute to institute. Hence The present study was conducted to determine the prevalence and antibiogram ESBL producing E.coli and K. pneumoniae isolates obtained from different clinical isolates, from different hospitals in Khartoum State, Sudan, using phenotypic laboratory methods.

MATERIALS AND METHODS

Study: Design and Area

This is analytical –comparative A cross-sectional study conducted over January –December 2017 in [4] Hospitals, Kartoum State, Sudan. Namely: Soba University Hospital, Omdurman Teaching Hospital, Royal care and Fedail hospitals.

Study: Population and Sampling

The study population included all *E. coli* and *K. pneumoniae*, that were isolated from different clinical specimen during routine examination allover study period in the (4) study hospitals.

The sample size was determined using the formula advanced by Kish and Leslie [9]. Basing on results of a previous study in a similar setting, a prevalence of 40 % [10] and confidence interval of 95 %, marginal error (5%) were used in the formula. The sample size was estimated to be 368 isolates. Isolates data regarding patient (age, sex, hospital department and source), were collecting from record using data collection sheet. Judgment sampling method was used to include isolates until the required sample size was fulfilled.

Isolates: Identity Confirm, Purity and Preservation

Isolates were transported to Central Public Health Laboratory –Khartoum State where all phenotypic tests were had been done. Single colonies were selected and confirmed by standard bacteriological technique [11, 12].

Colonies inoculated by streaking on MacConkey agar media (Lab M, UK) for the confirm identity of *E. coli* and *K.pneumoniae*, fter 24hr incubation , isolates were further characterized using conventional/standard microbiology techniques (colonial morphology, Gram-staining, catalase test , indole test, citrate utilization test, Voges-Proskauer test, methyl red test and urease test and . Purified bacterial cultures stocked in nutrient broth with 20% glycerol and stored at –80°C until use.

ESBL Screening by Disc Diffusion Test

Screening test for ESBL detection was done by disc diffusion test (Kirby-bauer), according to the Clinical and Laboratory Standard Institute [13], using Standard inoculums adjusted to 0.5 McFarland were swabbed onto Muller-Hinton agar (MHA) (Hi-media), after 24hr incubation Isolates showing inhibition zone size of≤22 mm with ceftazidime (30 µg), ≤ 25 mm with ceftriaxone (30 µg), and≤27 mm with cefotaxime (30 µg) were interpreted as screening test positive for ESBL production. The antibiotics discs and MHA were all taken from HiMedia Laboratories (India).

ESBL Confirmation by Double Disc Synergy Test (DDST) Method

In DDST, Plates were inoculated for routine drug susceptibility using the modified Kirby-Bauer standardized disc diffusion method. Ceftazidime (30 µg) and ceftriaxone (30 µg) discs were placed on either side of co-amoxiclav (20 + 10 µg) 15 mm apart on MHA plate inoculated with standardized McFarland's standard inoculum, ESBL-positive strains showed inhibition zone size around the antibiotic disc above 5 mm in the presence of co-amoxiclav (20 + 10 µg). This expansion occurred because the clavulanic acid present in the Augmentin disc inactivated the ESBL produced by the test organism [14].

Antibiotics Susceptibility Testing for ESBL Confirmed Isolates:

Antimicrobial susceptibility testing was performed using agar disk diffusion method according to CLSI recommendations [13]. The antimicrobial disks were tested (antibiotic concentration in µg) ceftazidime (CAZ: 30µg), cefotaxime (CTX: 30µg), Trimethoprim-sulfamethoxazole (SXT: 1.25/ 23.75 µg), gentamicin (GM: 10µg), amikacin (AN: 30µg), imipenem (IMP: 10µg), and meropenem (MEM; 10µg), Amoxicillin-clavulanate (AMC:20/10 µg) , Ciprofloxacin (CIP: 5 µg), Nitrofurantoin (NIF:300 µg) Each of the isolate was standardized to 0.5 McFarland equivalents and aseptically inoculated on prepared Muller-Hinton agar plates using sterile swab stick. The inoculated plates were allowed to stand for 10 min-15 min were placed on the inoculated plates using sterile forceps. The plates were incubated at 37°C for 24 h after which the zones of inhibition around each disc were measured, recorded and interpreted according to the CLSI guidelines. The

laboratory investigation was carried out at Central Public Health Laboratory Khartoum State –Sudan.

Quality Control

Standard strain (*E. coli* ATCC 25922™) was used to check for phenotypic ESBL testing. Moreover, *E. coli* (ATCC25922) and P. aeruginosa (ATCC 27853) were used to check the potency of antimicrobial discs and to control drug susceptibility testing procedures.

Ethics Considerations

Ethical approval was not required to carry out this work as the bacterial isolates were collected as part of routine patient care investigation in the hospital. Consent had been taken from Khartoum ministry of Health to conduct this study.

Data Analysis

Data were analyzed using Statistical package for Social Sciences version 20 Software (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp). The Chi-square test was employed to compare the association of categorical variables. P-value of less than 0.05 was considered as statistically significant.

RESULTS

Isolates Characteristics

Overall, (368) isolates took part in this study. Confirm Identity of isolates based on their growth characteristics on MacConkey agar, and biochemical tests profile results. The following tables and figure presented the characteristics of these isolates.

Frequency of study isolates presented on Table-1. While Demographic characteristics of study isolates illustrated on Figure-1.

Majority of study isolates was *E. coli* (58.7%, n=216) (Table-1). Majority of study isolate obtained from female (53.5% n=197), the predominant of these isolates were obtained from the age group (21-45) year (43.7%, n=86) (Figure-1).

The predominant study isolates were collected from Soba University Hospital (34.2%, n=126), urinary isolates in this study represent predominate of study isolates (42.7%, n= 157), and the highest frequency of study isolates were obtained from Obstetrics & Gynecology (OBS/GYN) departments (31%, n=114), the predominant (OBS/GYN) isolate was *E. coli* (75%, n=86) (Table-3).

Prevalence of ESBL phenotype

The overall ESBL-EK prevalence among the (4) hospitals was (36.7%, n= 135). ESBL phenotype prevalence among *E. coli* isolates was (34.3%, n= 74), while among *K. pneumoniae* isolates was (40.1%, n=61). (Table-3) illustrated Demographic and clinical characteristics of the study isolates in association with ESBL production.

Statistically significant associations was observed between ESBL-producer and non-ESBL –in regard to age groups (p< 0.0001) , the highest proportion of ESBL-phenotype isolates was detected among age group (21-45) yrs (51.4%,73/142), However, there were no significant association between ESBL-producers and non-ESBL producer and gender of patients, hospital of admission. For instance, both Omdurman Teaching and Soba University hospitals reported the highest prevalence of ESBL.EK (40.2 %; 33/82 vs 39.7%; 50/126) respectively (Table-3).

Table -1: Frequency of study isolates

Isolate	Frequency	%
E. coli	216	58.7
K. pneumoniae	152	41.3
Total	368	100.0

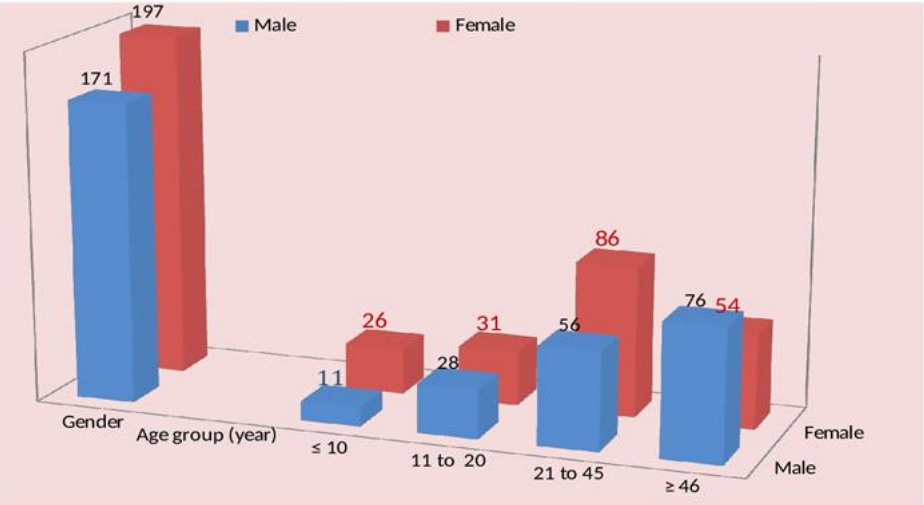

Fig-2: Demographic characteristics of study isolates

Table-2: Clinical characteristics of the study isolates

Variable	*E.coli*; n (%)	*K. pneumoniae*	Total; n (%)
Hospitals			
Soba	72 (57. 1)	54	126
Omdurman	48 (58.5)	34	82
Royal care	58 (59.8)	39	97
Fedail	38 (55.9)	25	63
Hospitals Departments			
Obstetrics &gynecology	86(75.4)	28	114(31).
Medicine	33(47.8)	36	69(18.8)
Intensive care	21(45.7)	25	46(12.5)
Outpatients	39(54.9)	32	71(19.3)
Other	37(54.4)	31	68(18.5)
Specimen type			
Urine	127	30	157(42.7)
Swabs	28	24	52(14.1)
Pus	16	28	44(12)
Sputum	6	28	34(9.2)
Blood	14	13	27(7.3)
body fluids	8	6	14(3.8)
Others	17	23	40(10.9)

Table-3: Demographic and clinical characteristics of the study isolates in association with ESBL production

Variable	ESBL Producer n (%)	Non ESBL producer	P.value and interpretation
Gender			0.448
Male	59 (34.5)	112	not significant
Female	76 (38.5)	121	
Age groups			
< 10	01 (2.7)	36	
(11-20)	20 (33.9)	39	< 0.0001
(21-45)	73 (51.4)	69	highly significant
>46	41 (31.5)	89	
Hospitals			
Soba	50 (39. 7)	76	0.493
Omdurman	33 (40.2)	49	Not significant
Royal care	30 (30.9)	67	
Fedail	22 (34.9)	41	
Hospitals Departments			
Obstetrics &gynecology	44(38.2)	71	0.283
Medicine	24(34.3)	46	Not significant
Intensive care	23(50)	23	
Outpatients	22(31.9)	47	
Other	22(32.4)	46	
Specimen type			
Urine	48(30.6)	109	0.006
Swabs	20(45.5)	24	significant
Pus	29(55.8)	23	
Sputum	13(38.2)	21	
Blood	11(40.7)	16	
body fluids	01(7.1)	13	
Others	13(32.5)	27	

Statistically significant association was noted between ESBL-producer and non-ESBL-producer with anatomically infected sites (P = 0.006). For instance, from pus and swabs, 55.8% and 45.5% of isolates were ESBL-producer, respectively, while Urinary isolates with ESBL phenotype were the most frequent ESBL phenotype 35.6% (n=48), However, there were no significant association between ESBL-producers and non-ESBL producer and hospital department, regarding proportion of ESBL in hospital departments, the ESBL phenotype proportion ranged between (50%) in Intensive Care Units and (25%) in Pediatrics departments (Table-3).

Antimicrobial Resistance among ESBL-Producing Isolates

Meropenem and imipenem were the most active antimicrobial agents against all the ESBL phenotype isolates (resistant % less than 5%), with statistically no significant different between ESBL-EC and ESBL –KP. for both antibiotics (P.value >0.05). The highest proportions of overall resistance were observed for ampicillin (100%), cefotaxime (87.4%) and ceftazidime (90.4%) (Figure-2).

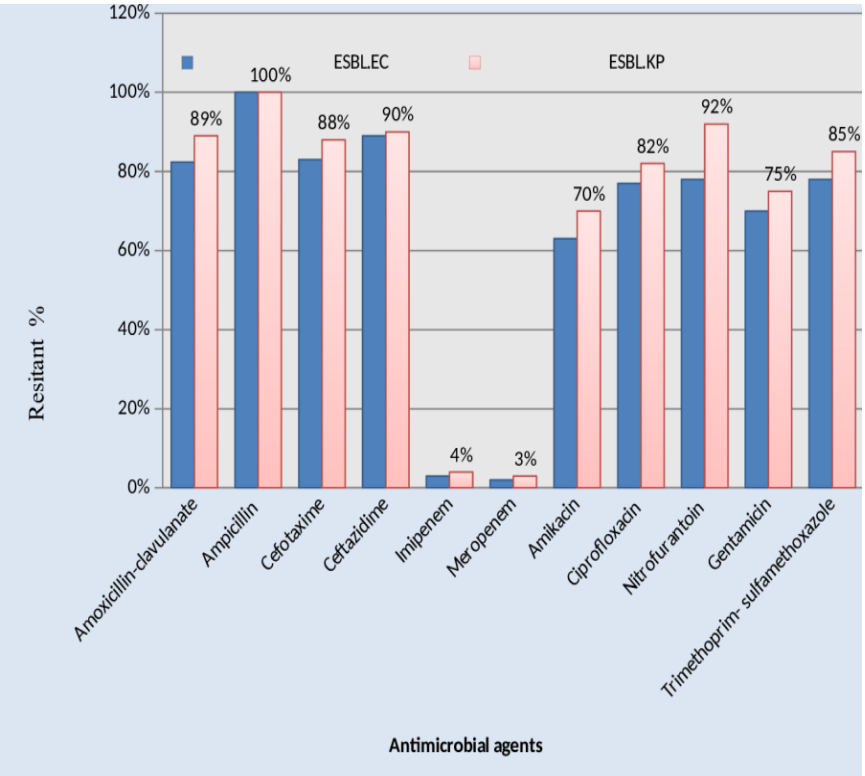

Fig-2: Antimicrobial resistant pattern of ESBL phenotype isolates

Among ESBL-EC and ESBL-KP resistance to ciprofloxacin (indicating cross-resistance to all fluoroquinolones) was found in (77.0%) and (81.9%), respectively. Resistance to co-trimoxazole was observed in (80%) of ESBL-EC and (88%) of ESBL-KP.

According to the definitions of (MDR) by being non susceptible to ≥1 agent in more than (3) antimicrobial categories [15], all of ESBL phenotype isolates in this study (n=135) were MDR.

DISCUSSION

In this study ,The overall ESBL-EK prevalence among the (4) hospitals was (36.7%), ESBL phenotype prevalence among *E. coli* isolates was (34.3%), while among *K. pneumoniae* isolates was (40.1%). The current study findings is consistent with other studies conducted in Sudan, that revealed the prevalence of ESBL-producing isolates varied among hospitals (18.2% to 45.1%), although a high prevalence was recorded as (45.1%) at Khartoum Teaching Hospital [10]. However, other published study in Khartoum indicated very high prevalence of ESBL Phenotype in comparison to current study that was (53%) among MDR *E. coli* and *Klebsiella* species , this can be explained by the targeted isolates in that study is MDR and only from urine [16]. Other greater ESBL prevalence in comparison to current study reported from Radiation and Isotopes Centre of Khartoum found that proportions of extended-spectrum β-lactamase producing isolates from cancer patients, were (49.2%) [17]. This difference reflected the immunity and other risk factors for ESBL contributing patients participating in that study.

Regarding prevalence outside Khartoum State, this study reported low prevalence in comparison to recently study from White Nile State ,stated very high ESBL phenotype prevalence that was (83.3%) [18], this high difference was contributed to both physicians and patients influence patterns of antibiotics use, which vary by some socioeconomic characteristics of areas and of individuals and families. However current study finding was consistent with other recently study done at Port Sudan Teaching Hospital, in which ESBL phenotype was (40.4%) [19].

The current study findings were in harmony with two countries known with highest ESBL that are India and Pakistan. In India it ranged from (22 to 75%), in Pakistan (40%) [20-22]. These findings rank Sudan with the highest ESBL producer countries, which is alarming indicator.

In comparing this study finding with the neighboring countries, this study finding in agreement with Ethiopia and Egypt, while little better than Kenya and Uganda. In Kenya the median ESBL proportion was 45.8 % (13.1–88.3). Ethiopia recorded median ESBL proportion of 30.9 % (21.9–40.4). In Uganda the recorded median ESBL proportion was 61.7 % (43.8–81.4), in Egypt 38.5% [23, 24].

Comparing the findings with this prevalence from European countries ,ESBL prevalence is much higher than that reported from Italy (6.3 %), Greece (27.4 %), Netherlands (2.0 %) and Germany (2.6%) [25]. The high prevalence has been significantly revealed in low and middle income countries. This infers that the prevalence of ESBL may have an association with the financial status of a general public, it may also explained by simple access to antimicrobial agents in the study area.

The susceptibility pattern of the ESBL-producing isolates in this study indicates a cross resistance to many other common antibiotics and Carbapenems were the most efficacious drugs; imipenem and meropenem showed 80% to 100% sensitivity. This finding in agreement to many other studies [26-28]

CONCLUSION

In this study, there is a high rate of ESBL-EK and MDR that may be attributed to the extensive inappropriate use of third generation cephalosporins. Surveillance of multidrug-resistant among different pathogenic bacteria is essential to guide the empirical treatment strategies for these infections in Sudan. There is a strong need to carry out further molecular studies to delineate the prevailing types of ESBL in Sudan.

ACKNOWLEDGEMENTS

The authors greatly appreciate prof. Assoc. Prof." Ahmed Mohammed Ahmed for supporting this study given me valuable advices throughout this work. I also grateful to the Mr. Nureldin Mohammed Ali (Head of the Central Public Health Laboratory - Laboratories Administration -Khartoum State), for providing me the infrastructure support to conduct all my study phenotypic experiments in the department's laboratory. Deep gratitude goes to all colleagues in Central Public Health Laboratory, Omdurman teaching, soba university, Royal care and Fedial hospitals for their cooperation during my study period.

REFERENCES

1. Deodurg, P. M., Sureka, R. K., & Mala, R. D. (2014). Prevalence and antibiogram of extended spectrum β-lactamase producing *Klebsiella pneumoniae* in a tertiary care hospital. *Journal of Scientific and Innovative Research*, *3*(2), 155-159.
2. Margulieux, K. R., Srijan, A., Ruekit, S., Nobthai, P., Poramathikul, K., Pandey, P., ... & Swierczewski, B. E. (2018). Extended-spectrum β-lactamase prevalence and virulence factor characterization of enterotoxigenic *Escherichia coli* responsible for acute diarrhea in Nepal from 2001 to 2016. *Antimicrobial Resistance & Infection Control*, *7*(1), 87.
3. Andrew, B., Kagirita, A., & Bazira, J. (2017). Prevalence of extended-spectrum beta-lactamases-producing microorganisms in patients admitted at KRRH, Southwestern Uganda. *International journal of microbiology*, *2017*.
4. Rawat, D., & Nair, D. (2010). Extended-spectrum β-lactamases in Gram Negative Bacteria. *Journal of global infectious diseases*, *2*(3), 263.
5. Wu, U. I., Chen, W. C., Yang, C. S., Wang, J. L., Hu, F. C., Chang, S. C., & Chen, Y. C. (2012). Ertapenem in the treatment of bacteremia caused by extended-spectrum beta-lactamase-producing *Escherichia coli*: a propensity score analysis. *International Journal of Infectious Diseases*, *16*(1), e47-e52.
6. Paterson, D. L., & Bonomo, R. A. (2005). Extended-spectrum β-lactamases: a clinical update. *Clinical microbiology reviews*, *18*(4), 657-686.
7. Cantón, R., González-Alba, J. M., & Galán, J. C. (2012). CTX-M enzymes: origin and diffusion. *Frontiers in microbiology*, *3*, 110.
8. Muthupandian, S., Balajee, R., & Barabadi, H. (2017). The prevalence and drug resistance pattern of extended spectrum β–lactamases (ESBLs) producing Enterobacteriaceae in Africa. *Microbial pathogenesis*.
9. Kateregga, J. N., Kantume, R., Atuhaire, C., Lubowa, M. N., & Ndukui, J. G. (2015).

Phenotypic expression and prevalence of ESBL-producing Enterobacteriaceae in samples collected from patients in various wards of Mulago Hospital, Uganda. *BMC pharmacology and Toxicology*, *16*(1), 14.

10. Ibrahim, M. E., Bilal, N. E., Magzoub, M. A., & Hamid, M. E. (2013). Prevalence of extended-spectrum β-lactamases-producing *Escherichia coli* from Hospitals in Khartoum State, Sudan. *Oman medical journal*, *28*(2), 116.
11. Cheesbrough, M. (2006). *District laboratory practice in tropical countries*. Cambridge university press.
12. McCartney, J. E., Collee, J. G., & Mackie, T. J. (1989). *Practical medical microbiology*. Charchil Livingstone.
13. Wayne, P. A. (2011). Clinical and laboratory standards institute. Performance standards for antimicrobial susceptibility testing.
14. Iqbal, R., Ikram, N., & Shoaib, M. (2017). Phenotypic cofirmatory disc diffusion test (PCDDT), double disc synergy test (DDST), E-test OS diagnostic tool for detection of extended spectrum beta lactamase (ESBL) producing Uropathogens. *J Appl Biotechnol Bioeng*, *3*(3), 344-349.
15. Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., ... & Paterson, D. L. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical microbiology and infection*, *18*(3), 268-281.
16. Mekki, A. H., Hassan, A. N., & Elsayed, D. E. M. (2010). Extended spectrum beta lactamases among multi drug resistant *Escherichia coli* and Klebsiella species causing urinary tract infections in Khartoum. *African Journal of Bacteriology Research*, *2*(3), 18-21.
17. Nurain, A. M., Bilal, N. E., & Ibrahim, M. E. (2015). The frequency and antimicrobial resistance patterns of nosocomial pathogens recovered from cancer patients and hospital environments. *Asian Pacific Journal of Tropical Biomedicine*, *5*(12), 1055-1059.
18. Ahmed, Y. M. A., Mohammed El Imam, M., Khalid, K. E., Mohamed, B. A., & Elhag, K. M. (2014). Phenotype and genotype of extended-spectrum-beta-lactamases in Sudanese patients with UTI.
19. Mohammed, I. K. (2016). *Phenotypic Detection of Extended Spectrum β–Lactamases in Gram Negative Bacilli among Hospitalized Pregnant Women-Omdurman* (Doctoral dissertation, Sudan University of Science & Technology).
20. Agrawal, P., Ghosh, A. N., Kumar, S., Basu, B., & Kapila, K. (2008). Prevalence of extended-spectrum β-lactamases among *Escherichia coli* and *Klebsiella pneumoniae* isolates in a tertiary care hospital. *Indian Journal of Pathology and Microbiology*, *51*(1), 139.
21. Abhilash, K. P., Veeraraghavan, B., & Abraham, O. C. (2010). Epidemiology and outcome of bacteremia caused by extended spectrum beta-lactamase (ESBL)-producing *Escherichia coli* and Klebsiella spp. in a tertiary care teaching hospital in south India. *J Assoc Physicians India*, *58*(Suppl), 13-17.
22. Abrar, S., Hussain, S., Khan, R. A., Ain, N. U., Haider, H., & Riaz, S. (2018). Prevalence of extended-spectrum-β-lactamase-producing Enterobacteriaceae: first systematic meta-analysis report from Pakistan. *Antimicrobial Resistance & Infection Control*, *7*(1), 26.
23. Sonda, T., Kumburu, H., van Zwetselaar, M., Alifrangis, M., Lund, O., Kibiki, G., & Aarestrup, F. M. (2016). Meta-analysis of proportion estimates of Extended-Spectrum-Beta-Lactamase-producing Enterobacteriaceae in East Africa hospitals. *Antimicrobial Resistance & Infection Control*, *5*(1), 18.
24. Fadil, A. A., Hakeem, M. A., & Abdelraheem, A. R. (2017). ESBL-Producing *E. coli* and Klebsiella among Patients Treated at Minia University Hospitals. *Journal of infectious diseases & preventive medicine*, *5*(2), 1000156.
25. Bouchillon, S. K., Johnson, B. M., Hoban, D. J., Johnson, J. L., Dowzicky, M. J., Wu, D. H., ... & Bradford, P. A. (2004). Determining incidence of extended spectrum β-lactamase producing Enterobacteriaceae, vancomycin-resistant Enterococcus faecium and methicillin-resistant Staphylococcus aureus in 38 centres from 17 countries: the PEARLS study 2001–2002. *International journal of antimicrobial agents*, *24*(2), 119-124.
26. Soltani, R., Ehsanpoor, M., Khorvash, F., & Shokri, D. (2014). Antimicrobial susceptibility pattern of extended-spectrum β-lactamase-producing bacteria causing nosocomial urinary tract infections in an Iranian referral teaching hospital. *Journal of research in pharmacy practice*, *3*(1), 6.
27. Roshan, M., Ikram, A., Mirza, I. A., Malik, N., Abbasi, S. A., & Alizai, S. A. (2011). Susceptibility pattern of extended spectrum ß-lactamase producing isolates in various clinical specimens. *J Coll Physicians Surg Pak*, *21*(6), 342-6.
28. Hassan, R. M., & Hammad, M. N. Prevalence and Antimicrobial Susceptibility Pattern of Extended Spectrum β-Lactamases Producing *Escherichia coli*, *Klebsiella pneumoniae* and Proteus mirabilis in Khartoum Sudan.